

University of Vermont
ScholarWorks @ UVM

Graduate College Dissertations and Theses

Dissertations and Theses

6-24-2008

Influence of Altered Temperature and Precipitation on Desert Microfauna and Their Role in Mediating Soil Nutrient Availability

Brian Darby
University of Vermont

Follow this and additional works at: <http://scholarworks.uvm.edu/graddis>

Recommended Citation

Darby, Brian, "Influence of Altered Temperature and Precipitation on Desert Microfauna and Their Role in Mediating Soil Nutrient Availability" (2008). *Graduate College Dissertations and Theses*. Paper 64.

This Dissertation is brought to you for free and open access by the Dissertations and Theses at ScholarWorks @ UVM. It has been accepted for inclusion in Graduate College Dissertations and Theses by an authorized administrator of ScholarWorks @ UVM. For more information, please contact donna.omalley@uvm.edu.

INFLUENCE OF ALTERED TEMPERATURE AND
PRECIPITATION ON DESERT MICROFAUNA AND THEIR ROLE IN
MEDIATING SOIL NUTRIENT AVAILABILITY

A Dissertation Presented

by

Brian J. Darby

to

The Faculty of the Graduate College

of

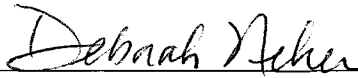
The University of Vermont

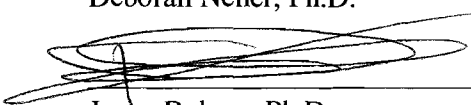
In Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
Specializing in Plant and Soil Science

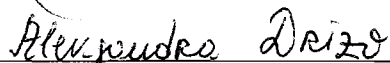
October, 2008


Accepted by the Faculty of the Graduate College, The University of Vermont, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, specializing in Plant and Soil Science.

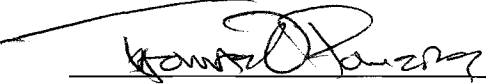
Dissertation Examination Committee:

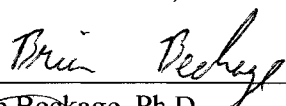

Deborah Neher, Ph.D. Advisor

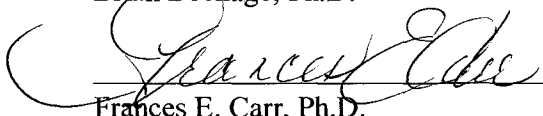

Jayne Belnap, Ph.D.


Aleksandra Drizo, Ph.D.


Donald S. Ross, Ph.D.


Thomas O. Powers, Ph.D.


Brian Beckage, Ph.D. Chairperson


Frances E. Carr, Ph.D. Vice President for Research and
Dean of the Graduate College

Date: May 13, 2008

Abstract

Arid-land deserts comprise up to 30% of global land surface area and experience degradation by anthropogenic land use demands. Sixteen of the 19 climate models used by the International Panel on Climate Change predict increased temperature and prolonged periods of drought for much of the arid southwest US. Soil microfauna consume organic-bound nitrogen and excrete soluble inorganic and dissolved organic nitrogen that is available for plant uptake or as substrate for microbial saprotrophs. However, the growth and activity of nematodes and protozoa is restricted to periods of adequate temperature and moisture. The objective of this dissertation research project is to determine the possible effects of predicted climate changes on cool desert soil nematodes and protozoa and their role in nitrogen cycling. Climate changes are predicted to affect nematodes more adversely than protozoa. Nematodes are thought to contribute more to nitrogen cycling through dissolved organics while protozoa are thought to contribute relatively more to nitrogen cycling through inorganic nitrogen. A shift in community composition favoring amoebae over nematodes could shift the relative balance of organic and inorganic pools of soil nitrogen. Soil microfauna are not readily observed in their soil habitat, so extraction and enumeration techniques are adapted and tested for the desert microfaunal communities. Desert soil nematodes and protozoa participate in energy channels deriving from vascular and non-vascular primary producers as well as bacterial and fungal saprotrophs. Environmental conditions influence the prey that are available to nematodes and protozoa and, thus, indirectly affect the relative composition of microfaunal feeding groups. Environmental conditions also directly affect microfauna. Not only are nematodes affected more negatively by adverse abiotic stress than amoebae, many species of bacterivorous nematodes appear to be susceptible to unique combination of the major abiotic stresses experienced in the desert. Findings from a two-year field experiment treated with elevated temperature and summer precipitation are consistent with lab and field mesocosm experiments, but highlight the uncertainty inherent in predicting long-term trends with brief experiments. In comparison with temperature and precipitation, long-term elevated carbon-dioxide enrichment was not shown to affect the abundance of desert soil microfauna directly but did affect the distribution of protozoa and the composition of nematodes indirectly through altered plant water use patterns. Continued work is needed to devise experimental systems that quantify the relative role of microfaunal functional groups in nutrient cycling.

Acknowledgements

Many thanks go to my advisor, Dr. Deborah Neher, whose enduring patience and encouragement motivated me through this project. You balanced enthusiasm with diligence and helped me to understand the process of learning. I thank my committee members for offering their time and expertise to my growth as a student and scientist: Dr. Brian Beckage, Dr. Jayne Belnap, Dr. Aleksandra Drizo, Dr. Tom Powers, and Dr. Don Ross. I also thank the fellow lab members who have supplied helpful conversations and assistance in the lab.

This dissertation research is supported by a grant from the Department of Energy, Program for Ecosystem Research, awarded to Jayne Belnap (USGS, Moab, Utah), Cheryl Kuske (Los Alamos National Laboratory, New Mexico), and Deborah Neher (University of Vermont, Burlington, VT). The research would not have been possible without the hard work and expertise of the USGS field crew in Moab, UT, and I especially appreciate the assistance of Dr. David Housman as a valuable point of contact for many years.

Awards from the Soil Ecology Society, University of Vermont Graduate Student Association and College of Arts and Sciences, Dow Agrosciences, Soil Science Society of America, and International Society of Protistologists made travel to conferences possible.

Finally, I thank the dear friends I've made in Vermont, the fellow graduate students who have helped me to enjoy the time, my extended family, grandparents, and siblings, and to my Mom and Dad, Carl and Donna, for their interest, encouragement and example.

Table of Contents

Acknowledgements	ii
List of Tables	vi
List of Figures	vii
Chapter 1. Introduction	1
Desert Soil Environment	1
Desert Soil Microfauna	2
Abiotic Stress.....	3
Nitrogen Transformations	5
From Principles to Predictions.....	8
References	10
Chapter 2. General Methodology.....	18
Methods	20
Protozoa Extraction	20
Nematode Extraction	21
Auxiliary Experiments.....	24
Results	26
Discussion.....	27
Protozoa Methods.....	27
Nematode Methods.....	28
References	30
Chapter 3. Energy Pathways in the Desert Soil Food Web.....	40
Methods	42
Plant Experiment	42
Crust Removal Experiment.....	43
Analysis	44
Results	47
Plant Experiment	47

Crust Removal Experiment.....	48
Discussion.....	49
References.	52
Chapter 4. Environmental Stress as a Determinant of Soil Microfaunal	
Composition	63
Methods	66
A. Wetting Frequency	67
B. Wetting Temperature	67
C. Wetting Season	68
D. Rapid Desiccation.....	69
E. Extreme Temperature	70
Statistical Analysis	71
Results	72
A. Wetting Frequency	72
B. Wetting Temperature	72
C. Wetting Season	73
D. Rapid Desiccation.....	73
E. Extreme Temperature.....	74
Discussion.....	74
References	78
Chapter 5. Field Test of the Effect of Increased Summer Temperature and	
Precipitation on Soil Microfauna.....	92
Methods	95
Experimental Design	95
Statistical Analysis	99
Food Web Modeling.....	101
Results	102
Discussion.....	105
References	111

Chapter 6. Indirect Effects of Elevated Carbon Dioxide on Desert Soil Nematodes and Protozoa.....	133
Methods	135
Analysis	137
Results	138
Discussion.....	139
References	142
Chapter 7. Interactions between Desert Nematodes and Protozoa	152
Methods	155
Microcosm Construction	155
Experiment 1. Nematode Addition.....	156
Experiment 2. Nematode Removal	157
Destructive Harvest and Analysis	157
Results	158
Experiment 1. Nematode Addition.....	158
Experiment 2. Nematode Removal	158
Discussion.....	159
References	160
Chapter 8. Comprehensive Bibliography	170

List of Tables

Table 1. Nematode Activity by Depth.	33
Table 2. Nematode Rehydration after Wetting Event.	34
Table 3. Nematode Specific Gravity.	35
Table 4. Treatment Analysis of Microfauna from Crust Removal Experiment.	55
Table 5. Depth Analysis of Microfauna from Crust Removal Experiment.	56
Table 6. Treatment Analysis of Indices from Crust Removal Experiment.	57
Table 7. Depth Analysis of Indices from Crust Removal Experiment.	58
Table 8. Analysis of Microfauna from Wetting Frequency Experiment.	80
Table 9. Growth Rates of Nematode Genera.	81
Table 10. Analysis of Microfauna from Wetting Temperature Experiment.	82
Table 11. Presence of Nematode Genera after Elevated Temperatures.	83
Table 12. Analysis of Microfauna from Winter Rapid Desiccation Experiment.	84
Table 13. Analysis of Microfauna from Summer Rapid Desiccation Experiment.	85
Table 14. Effect of Rapid Desiccation in Winter on Nematode Genera.	86
Table 15. Effect of Rapid Desiccation in Summer on Nematode Genera.	87
Table 16. Analysis of Microfauna from Extreme Temperature Experiment.	88
Table 17. Soil Chemistry at Field Site.	116
Table 18. Food Web Model Parameters.	117
Table 19. Analysis of Microfauna from Field Experiment.	118
Table 20. Nematode Genera Found at Field Site.	119
Table 21. Analysis of Nematode Composition from Field Experiment.	121
Table 22. Faunal Contributions to Nitrogen Cycling.	122
Table 23. Predation Rates of Omnivorous Desert Soil Fauna.	123
Table 24. Predation Rates of Bacterivorous Desert Soil Fauna.	124
Table 25. Intra-Annual Variability of Microfauna Populations and Composition.	125
Table 26. Analysis of Microfauna under Elevated Carbon Dioxide.	144
Table 27. Analysis of Nematode Communities under Elevated Carbon Dioxide.	145
Table 28. Analysis of Soil Properties under Elevated Carbon Dioxide.	146
Table 29. Cross-Desert Comparison of Nematode Genera.	147
Table 30. Analysis of Microfauna from Nematode Addition Experiment.	162
Table 31. Analysis of Inorganic Nitrogen from Nematode Addition Experiment.	163
Table 32. Analysis of Microfauna from Nematode Removal Experiment.	164
Table 33. Analysis of Inorganic Nitrogen from Nematode Removal Experiment.	165

List of Figures

Figure 1. Growth Rate of Desert Protozoa.....	14
Figure 2. Desert Protozoa in Elevated Temperature and Precipitation.	15
Figure 3. Conceptual Models of Nutrient Cycling in Fauna.....	16
Figure 4. Hypothesized Consequence of Climate Change on Soil Microfauna.....	17
Figure 5. Nematode Extraction Efficiency.	36
Figure 6. Nematode Specific Gravity.	37
Figure 7. Nematode Activity by Depth.....	38
Figure 8. Nematode Rehydration after Wetting Event.	39
Figure 9. Nematode Abundance and Composition around Plants.	59
Figure 10. Island in the Sky Nematode Community Composition around Plants.	60
Figure 11. Needles Nematode Community Composition around Plants.	61
Figure 12. Nematode Community Composition from Crust Removal Experiment.	62
Figure 13. Growth of Microfauna During Frequent Wetting.....	89
Figure 14. Growth of Nematodes with Elevated Temperature.	90
Figure 15. Nematode Biomass after Incubation at Various Temperatures.....	91
Figure 16. Data to Estimate Growth Potential Index.....	126
Figure 17. Observed Climate at Field Site.....	127
Figure 18. Seasonal Lamp Performance.	128
Figure 19. Diurnal Lamp Performance.	129
Figure 20. Growth of Microfauna through Two Experimental Years.	130
Figure 21. Microfauna Biomass under Elevated Temperature and Precipitation.	131
Figure 22. Nematode Diversity through Elevated Temperature and Precipitation.	132
Figure 23. Flagellates and Water Content under Elevated Carbon Dioxide.....	149
Figure 24. Ciliates and Water Content under Elevated Carbon Dioxide.....	150
Figure 25. Nematode Community Composition under Elevated Carbon Dioxide.	151
Figure 26. Nematode Biomass and Inorganic Nitrogen from Nematode Addition Experiment.	166
Figure 27. Protozoan Biomass from Nematode Addition Experiment.....	167
Figure 28. Nematode Biomass and Inorganic Nitrogen from Nematode Removal Experiment.	168
Figure 29. Protozoan Biomass from Nematode Removal Experiment.	169

CHAPTER 1. INTRODUCTION

Arid-land deserts comprise up to 30% of global land surface area and experience degradation from anthropogenic land use demands (Schlesinger et al., 1990). Sixteen of the 19 climate models used by the International Panel on Climate Change predict increased temperature and prolonged periods of drought for much of the arid southwest US (Seager et al., 2007). Soil microfauna transform organic-bound nitrogen into soluble inorganic and dissolved organic nitrogen that is available for plant uptake or as substrate for microbial saprotrophs. Amoebae and bacterivorous nematodes together account for about 30 % of the nitrogen mineralization in a shortgrass prairie (Hunt et al., 1987), but their activity in arid land soil is restricted to periods of adequate temperature and moisture. The objective of this dissertation research project is to predict the effect of altered temperature and precipitation on desert microfauna and their effect on soil nitrogen cycling. This introductory chapter reviews the relevant existing information and proposes an initial prediction based on current food web theory. The remaining chapters proceed to test elements of the prediction.

Desert Soil Environment

Winter-rain deserts, like the Colorado Plateau or Great Basin of North America, receive a majority of their annual precipitation during winter. In contrast, summer-rain deserts, such as the Sonoran, Chihuahuan, or Mojave Desert of North America, receive a majority of their precipitation during the summer. Biological activity in the desert is stimulated by rain events, so the thermal stress on biota during summer monsoons can be

more stressful than during winter rains. Climate models predict an increase in temperature for much of the arid southwest (Schlesinger et al., 1990). Climate models also predict various altered precipitation patterns, but the magnitude and direction of change in precipitation amount and frequency is still debated (Weltzin et al., 2003).

Perhaps the most distinctive feature of these soils is the biological soil crust that can represent up to 70% of the living vegetative soil cover. Early-colonizing fungi and cyanobacteria stabilize the soil surface and facilitate colonization by lichens, green algae, and mosses. Biological soil crusts increase the physical stability of surfaces and soil fertility through dust entrapment, photosynthesis, nitrogen fixation, and mineral chelation (reviewed in Belnap, 2003). The autotrophic components of crusts are highly sensitive to surface disturbances and climate changes, such as altered temperature and precipitation (Belnap, 2003). For example, rapid wetting and drying cycles reduce quantum yield, chlorophyll, and UV protective pigments of soil lichens (Belnap et al., 2004), and high temperatures ($>26^{\circ}\text{C}$) inhibit nitrogen fixation (Belnap, 2002).

Desert Soil Microfauna

Diverse soil fauna inhabit biological soil crusts and underlying soil (Neher et al., 2003). Earthworms, enchytraeids, and flatworms are generally absent from arid soils, but ants, spiders, scorpions, and microarthropods, like mites and collembolans, inhabit the surface and air-filled spaces of the desert soil. This study considers only the fauna, hereafter termed *microfauna*, which inhabit the water-filled pores of the soil. Desert microfauna become active during rain events. Water-film surfaces are ephemeral in the desert, so microfauna tolerate desiccation by entering a temporary dormant state called

anhydrobiosis for nematodes, tardigrades, and rotifers, or encystment for protozoa. The nematodes of this community with open buccal cavities (no stylet) ingest heterotrophic bacteria. The stylet-bearing nematodes in this community pierce plant roots, cyanobacteria, green algae, fungi, moss, and even other nematodes, depending on the size, shape, and aperture of their stylets (Yeates et al., 1993). Tardigrades pierce and suck cytoplasm from filamentous cyanobacteria, fungi, and moss. Rotifers feed on bacteria and particulates by filtering soil water through ciliated corona. Protozoa are single-celled organisms classified in this study as amoebae, flagellates, or ciliates, based on their body form and method of motility. Amoebae phagocytize bacteria, algae, cyanobacteria, fungi, nematodes and other protozoa. Flagellates ingest small particles and bacteria. Ciliates feed on small particles, bacteria, flagellates, and small ciliates through a ciliated cytostome, similar to rotifers.

Abiotic Stress

In addition to biological interactions, such as competition and prey availability, the growth of nematodes and protozoa is limited to periods of optimal moisture and temperature. First, moisture restricts the periods when growth is possible. DeMeure et al. (1979a) estimated that anhydrobiotic coiling of desiccation-tolerant nematodes was maximal at -0.3 to -0.6 MPa, which translated for their soil into six to nine monomolecular layers of water. Nematodes in the Negev Desert increased in abundance within two to three days after an artificial wetting event, and this response was more pronounced with a 15 to 20 mm than 5 to 10 mm rain amendment (Steinberger and Sarig, 1993). In the Chihuahuan Desert, Whitford et al. (1981) found no significant diurnal changes in the

abundance of nematodes in either litter or soil but did observe rapid anhydrobiosis of nematodes in litter and gradual anhydrobiosis of nematodes in soil following a simulated rain event. Freckman et al. (1987), also in the Chihuahuan Desert, found no difference in total abundance of nematodes between artificially irrigated soils (monthly 25-mm rain events or weekly 6-mm rain events) and control (non-irrigated) soils. Steinberger et al. (1984) were surprised to find virtually no influence of simulated rainfall on nematode abundance (applied every third day during August). In contrast, protozoa were more abundant in wetted soils than non-wetted control soils only after six days of wetting, but not longer (Parker et al., 1984). Secondly, high temperature restricts the growth rate when growth is possible (Figure 1). Nematodes and protozoa are more abundant and diverse in cool deserts with winter rainfall than hot deserts with summer rainfall (Steinberger et al., 2001; Darby et al., 2006, 2007). Similarly, abundance and diversity of nematodes and protozoa tend to be greatest during the cool seasons (Freckman and Mankau, 1986; Steinberger and Loboda, 1991; Liang and Steinberger, 2001; Rodriguez-Zaragoza et al., 2005).

Laboratory and field experiments illustrate that in certain conditions, moisture may interact with temperature in a non-additive manner so that rain on a hot day may influence fauna differently than rain on a cool day. The survival of nematodes through desiccation is proportional to the time spent at >97.7 % relative humidity (Crowe and Madin, 1975; DeMeure et al., 1979b). Crowe and Crowe (1982) suggest that rapid desiccation of nematodes increases membrane rupture by inhibiting the production of glycerol and trehalose. High temperatures increase evaporation and accelerate drying,

thereby reducing survival of microfauna encysting or entering anhydrobiosis. The hypothesis that desert microfauna are susceptible to physiological stress when forced to be active following summer-rain events is supported by Freckman and Mankau (1986), who observed reduced nematode abundance after summer monsoon rains in the Chihuahuan Desert. Previously, we transplanted soil mesocosms from Colorado Plateau to Chihuahuan Desert and from Chihuahuan Desert to Sonoran Desert to imitate a shift from winter rain to summer rain and the associated increase in summer temperatures (Darby et al., 2006). Consistent with our original hypothesis, amoebae, flagellates and ciliates all decreased in abundance when moved from Colorado Plateau to Chihuahuan Desert, but increased or remained the same when moved from Chihuahuan Desert to Sonoran Desert (Figure 2). However, protozoa did not respond to experimental summer rain amendments at either location, which was inconsistent with our hypothesis. Part of this dissertation is designed to determine whether increased temperature, summer precipitation, or an interaction of the two is most responsible for the decline in abundance.

Nitrogen Transformations

Ammonium, the initial product of biological fixation, is either used by the biological nitrogen-fixing organism for the production of biomass (i.e., immobilized) or can be released extracellularly (i.e., mobilized) by outward diffusion (Dodds et al., 1995) or membrane rupture upon wetting and drying (Millbank, 1982). Mobilized mineral nitrogen, like ammonium, is available for plant uptake (Hawkes, 2003), nitrification (Johnson et al., 2005), denitrification (Peterjohn and Schlesinger, 1991), volatilization

(Schlesinger and Peterjohn, 1991), or leaching. Organic nitrogen as biomass (such as proteins) can be decomposed into amino acids by extracellular protease enzymes and passed into the cells of saprotrophic microbes. Most desert microfauna are well known to consume bacteria or fungi, depending on the morphology of the organisms' feeding apparatus. Soil fauna can consume nitrogen-fixing organisms directly, although the microfauna best suited to feed on filamentous cyanobacteria appear to be those that otherwise pierce fungal hyphae rather than ingest bacteria (Wood, 1973; Yeates et al., 1993; Birkemoe and Liengen, 2000). However, there have also been reports of soil mites rejecting cyanobacteria as prey options (Hubert and Lukesova, 2001) and concentrated *Microcoleus vaginatus* culture filtrate being used as a nematicide (Khan et al., 2005). Thus, microfaunal grazers may perceive a difference in palatability between nitrogen-fixing and non-nitrogen fixing cyanobacteria or their growth conditions.

Nitrogen ingested by a grazer from prey biomass is digested incompletely and will either be excreted as soluble or particulate organic waste or assimilated. Assimilated material is used to create biomass; carbon is mineralized into CO₂ through respiration and nitrogen is mineralized into NH₃ through catabolism and normal protein turnover (Figure 3). Carbon mineralization is expected to scale allometrically with biomass (West et al., 1997, 1999), and respiration (R, $\mu\text{l O}_2 \text{ h}^{-1}$) as a function of live biomass (mg) across a range of soil invertebrates was estimated to be $R = 0.357B^{0.813}$ (Ryszkowski, 1975). Wright (1975a) estimated that ammonia was 23 to 35 % of total nitrogen excreted by *Panagrellus redivivus*, a bacterivorous nematode, but noted that the organic urea (3 to 7%), amino acids (10 to 34%), and protein (42 to 35%) were also significant excretory

products. Wright (1975b) also demonstrated the existence of enzymes for a functional urea cycle and the incorporation of radiocarbon-labeled intermediates into urea. There is evidence for both accelerated protein turnover (Hawkins et al., 1987; Hawkins, 1991) and downregulated cellular metabolism (Hand and Hardwig, 1996; Guppy and Withers, 1999).

To overcome the technical challenges of measuring small quantities of nitrogen excretion in soil microfauna, Hunt et al. (1987) proposed a conceptual framework as a way to predict nitrogen mineralization rates by soil fauna (Figure 3). In this approach, a constant proportion of what is consumed is considered assimilated, and a portion of what is assimilated is retained as biomass, and the rest mineralized. Consumption, assimilation, and production efficiencies are estimated from available laboratory studies (e.g., Marchant and Nicholas, 1974) and applied to soil fauna populations to estimate the amount of prey that must be consumed to maintain observed steady-state biomass. The result was the model prediction that most of the nitrogen mineralization by soil fauna originates from the bacterivorous functional guilds like amoebae and bacterivorous nematodes. This observation reflects the theory that organisms consuming high nitrogen content prey like bacteria are more limited by carbon than nitrogen and are likely to excrete more nitrogenous wastes. Organisms consuming low nitrogen content prey like fungi are more limited by nitrogen than carbon and are likely to excrete less nitrogenous wastes.

From Principles to Predictions

Hunt and Wall (2002) address the question of “how many species does it take to maintain ecosystem function?” by comparing food web dynamics run to steady state after deleting each of the 15 functional groups, one at a time. They found that only two functional group deletions (i.e., bacteria and saprophytic fungi) resulted in the extinction of other groups and only three functional group deletions (i.e., bacteria, saprophytic fungi, and herbivorous nematodes) resulted in a 10% alteration in some index of ecosystem function (i.e., nitrogen mineralization or primary production). They concluded that “the results suggest that ecosystems could sustain the loss of some functional groups with little decline in ecosystem services, because of compensatory changes in the abundance of surviving groups.” However, I suggest that the wholesale loss of entire functional groups is an unlikely scenario from climate change predictions. The most likely changes appear to be subtle and sometimes idiosyncratic shifts in the relative composition of existing functional groups (Sohlenius and Bostrom, 1999; Todd et al., 1999; Convey and Wynn-Williams, 2002). Intolerant species lost from a functional group are often replaced by what appear to be functionally redundant species of a similar functional group (Todd et al., 1999; Bakonyi and Nagy, 2000). Rather than address the question “how many species does it take to maintain ecosystem function,” I ask, “what happens to ecosystem function after a directional shift in species and functional group composition?”

The primary literature does not make a clear prediction regarding the influence of altered climate change on desert soil fauna community composition and their role in soil

ecosystem functioning, such as nitrogen cycling. An initial impression might suggest that function is additive and a reduction in the abundance of any functional groups will lead to a reduction in the function of that group. A significant outcome of this research is an alternative perspective of how altered climate affects the role desert soil microfauna in ecosystem functioning (Figure 4). Here, nitrogen cycling is considered non-additive and the decline in abundance of stress-intolerant functional groups and species (Chapter 3) could be replaced in part by stress-tolerant functional groups and species. According to this scheme, the current cool desert climate is such that elevated temperature and summer precipitation represent an abiotic stress for most organisms we find in the arid lands of southwestern US. Nematodes are affected more negatively by these abiotic stresses than amoebae, and contrasting species of nematodes are affected differentially by abiotic stress (Chapter 3). Because nematode body size is an order of magnitude greater than amoebae, they are expected to respire less per unit of biomass than amoebae (West et al., 1997, 1999, Ryszkowski, 1975). Thus, nematodes contribute proportionately more to nitrogen cycling through dissolved organics (Wright, 1975a) while amoebae contribute more to nitrogen cycling through excretion of inorganic nitrogen (Hunt et al., 1987). Similarly, stress tolerant nematodes that over-produce cellular-protecting mechanisms may be linked with greater rates of nitrogen waste removal.

In sum, the proposed climate changes could decrease the abundance of nematodes more than of amoebae and shift the balance of nitrogen cycling by reducing the relative contributions of dissolved organics and increasing the relative contributions of labile inorganics. This is significant because ammonium, the form of inorganic nitrogenous

wastes by nematodes and protozoa, can be oxidized rapidly in this system (Johnson et al., 2005) and exported through leaching of nitrate (Johnson et al., 2007). Future research should be careful to compare the balance of organic and inorganic nitrogen in desert soils and consider the relative contributions of soil microfauna to these substrate pools.

References

- Bakonyi, G., & Nagy, P. (2000). Temperature- and moisture-induced changes in the structure of the nematode fauna of a semiarid grassland - patterns and mechanisms. *Global Change Biology*, 6(6), 697-707.
- Belnap, J. (2002). Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biology and Fertility of Soils*, 35(2), 128-135.
- Belnap, J. (2003). The world at your feet: Desert biological soil crusts. *Frontiers in Ecology and the Environment*, 1(4), 181-189.
- Belnap, J., Phillips, S. L., & Miller, M. E. (2004). Response of desert biological soil crusts to alterations in precipitation frequency. *Oecologia*, 141(2), 306-316.
- Birkemoe, T., & Liengen, T. (2000). Does collembolan grazing influence nitrogen fixation by cyanobacteria in the high arctic? *Polar Biology*, 23(8), 589-592.
- Convey, P., & Wynn-Williams, D. D. (2002). Antarctic soil nematode response to artificial climate amelioration. *European Journal of Soil Biology*, 38(3-4), 255-259.
- Crowe, J. H., & Crowe, L. M. (1982). Induction of anhydrobiosis - membrane-changes during drying. *Cryobiology*, 19(3), 317-328.
- Crowe, J. H., & Madin, K. A. C. (1975). Anhydrobiosis in nematodes: Evaporative water loss and survival. *Journal of Experimental Zoology*, 193, 323-334.
- Darby, B. J., Housman, D. C., Zaki, A. M., Shamout, Y., Adl, S. M., Belnap, J., et al. (2006). Effects of altered temperature and precipitation on desert protozoa associated with biological soil crusts. *Journal of Eukaryotic Microbiology*, 53(6), 507-514.
- Darby, B. J., Neher, D. A., & Belnap, J. (2007). Soil nematode communities are ecologically more mature beneath late- than early-successional stage biological soil crusts. *Applied Soil Ecology*, 35(1), 203-212.
- DeMeure, Y., Freckman, D. W., & Van Gundy, S. D. (1979). Anhydrobiotic coiling of nematodes in soil. *Journal of Nematology*, 11(2), 189-195.
- Demeure, Y., Freckman, D. W., & Van Gundy, S. D. (1979). *In vitro* response of four species of nematodes to desiccation and discussion of this and related phenomena. *Revue de Nématologie*, 2(2), 203-210.
- Dodds, W. K., Gudder, D. A., & Mollenhauer, D. (1995). The ecology of *Nostoc*. *Journal of Phycology*, 31(1), 2-18.

- Freckman, D. W., & Mankau, R. (1986). Abundance, distribution, biomass and energetics of soil nematodes in a northern Mojave Desert ecosystem. *Pedobiologia*, 29(2), 129-142.
- Freckman, D. W., Whitford, W. G., & Steinberger, Y. (1987). Effect of irrigation on nematode population-dynamics and activity in desert soils. *Biology and Fertility of Soils*, 3(1-2), 3-10.
- Guppy, M., & Withers, P. (1999). Metabolic depression in animals: Physiological perspectives and biochemical generalizations. *Biological Reviews*, 74(1), 1-40.
- Hand, S. C., & Hardewig, I. (1996). Downregulation of cellular metabolism during environmental stress: Mechanisms and implications. *Annual Review of Physiology*, 58, 539-563.
- Hawkes, C. V. (2003). Nitrogen cycling mediated by biological soil crusts and arbuscular mycorrhizal fungi. *Ecology*, 84(6), 1553-1562.
- Hawkins, A. J. S. (1987). Thermal responses reflect protein turnover in *Mytilus edulis* l. *Functional Ecology*, 1(4), 339-351.
- Hawkins, A. J. S. (1991). Protein-turnover - a functional appraisal. *Functional Ecology*, 5(2), 222-233.
- Hubert, J., & Lukesova, A. (2001). Feeding of the panphytophagous oribatid mite *Scheloribates laevigatus* (Acari : Oribatida) on cyanobacterial and algal diets in laboratory experiments. *Applied Soil Ecology*, 16(1), 77-83.
- Hunt, H. W., Coleman, D. C., Ingham, E. R., Ingham, R. E., Elliott, E. T., Moore, J. C., et al. (1987). The detrital food web in a shortgrass prairie. *Biology and Fertility of Soils*, 3, 57-68.
- Hunt, H. W., & Wall, D. H. (2002). Modelling the effects of loss of soil biodiversity on ecosystem function. *Global Change Biology*, 8(1), 33-50.
- Johnson, S. L., Budinoff, C. R., Belnap, J., & Garcia-Pichel, F. (2005). Relevance of ammonium oxidation within biological soil crust communities. *Environmental Microbiology*, 7(1), 1-12.
- Johnson, S. L., Neuer, S., & Garcia-Pichel, F. (2007). Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. *Environmental Microbiology*, 9(3), 680 to 689.
- Khan, Z., Park, S. D., Shin, S. Y., Bae, S. G., Yeon, I. K., & Seo, Y. J. (2005). Management of *Meloidogyne incognita* on tomato by root-dip treatment in culture filtrate of the blue-green alga, *Microcoleus vaginatus*. *Bioresource Technology*, 96(12), 1338-1341.
- Liang, W., & Steinberger, Y. (2001). Temporal changes in nematode community structure in a desert ecosystem. *Journal of Arid Environments*, 48(3), 267-280.
- Marchant, R., & Nicholas, W. L. (1974). An energy budget for the free-living nematode *Pelodera* (rhabditidae). *Oecologia*, 16, 237-252.
- Millbank, J. W. (1982). The assessment of nitrogen-fixation and throughput by lichens .3. Losses of nitrogenous compounds by *Peltigera membranacea*, *Peltigera polydactyla* and *Lobaria pulmonaria* in simulated rainfall episodes. *New Phytologist*, 92(2), 229-234.

- Neher, D. A., Walters, T. L., Tramer, E., Weicht, T. R., Veluci, R. M., Saiya-Cork, K., et al. (2003). Biological soil crust and vascular plant communities in a sand savanna of northwestern Ohio. *Journal of the Torrey Botanical Society*, 130(4), 244-252.
- Osler, G. H. R., & Sommerkorn, M. (2007). Toward a complete soil C and N cycle: Incorporating the soil fauna. *Ecology*, 88(7), 1611-1621.
- Parker, L. W., Freckman, D. W., Steinberger, Y., Driggers, L., & Whitford, W. G. (1984). Effects of simulated rainfall and litter quantities on desert soil biota - soil respiration, microflora, and protozoa. *Pedobiologia*, 27(3), 185-195.
- Peterjohn, W. T., & Schlesinger, W. H. (1991). Factors controlling denitrification in a Chihuahuan Desert ecosystem. *Soil Science Society of America Journal*, 55(6), 1694-1701.
- Rodriguez-Zaragoza, S., Mayzlish, E., & Steinberger, Y. (2005). Vertical distribution of the free-living amoeba population in soil under desert shrubs in the Negev Desert, Israel. *Applied and Environmental Microbiology*, 71(4), 2053-2060.
- Ryszkowski, L. (1975). Energy and matter economy of ecosystems. In W. H. van Dobben & R. H. Lowe-McConnel (Eds.), *Unifying concepts in ecology* (pp. 109-126). The Hague: Junk.
- Schlesinger, W. H., & Peterjohn, W. T. (1991). Processes controlling ammonia volatilization from Chihuahuan Desert soils. *Soil Biology & Biochemistry*, 23(7), 637-642.
- Schlesinger, W. H., Reynolds, J. F., Cunningham, G. L., Huenneke, L. F., Jarrell, W. M., Virginia, R. A., et al. (1990). Biological feedbacks in global desertification. *Science*, 247(4946), 1043-1048.
- Seager, R., Ting, M. F., Held, I., Kushnir, Y., Lu, J., Vecchi, G., et al. (2007). Model projections of an imminent transition to a more arid climate in southwestern North America. *Science*, 316(5828), 1181-1184.
- Sohlenius, B., & Bostrom, S. (1999). Effects of climate change on soil factors and metazoan microfauna (nematodes, tardigrades and rotifers) in a Swedish tundra soil - a soil transplantation experiment. *Applied Soil Ecology*, 12(2), 113-128.
- Steinberger, Y., Freckman, D. W., Parker, L. W., & Whitford, W. G. (1984). Effects of simulated rainfall and litter quantities on desert soil biota - nematodes and microarthropods. *Pedobiologia*, 26(4), 267-274.
- Steinberger, Y., Liang, W. J., Savkina, E., Meshi, T., & Barness, G. (2001). Nematode community composition and diversity associated with a topoclimatic transect in a rain shadow desert. *European Journal of Soil Biology*, 37(4), 315-320.
- Steinberger, Y., & Loboda, I. (1991). Nematode population dynamics and trophic structure in a soil profile under the canopy of the desert shrub *Zygophyllum dumosum*. *Pedobiologia*, 35(3), 191-197.
- Steinberger, Y., & Sarig, S. (1993). Response to soil nematode populations and the soil microbial biomass to a rain episode in the hot, dry Negev Desert. *Biol. Fertil. Soils*, 16, 188-192.
- Todd, T. C., Blair, J. M., & Milliken, G. A. (1999). Effects of altered soil-water availability on a tallgrass prairie nematode community. *Applied Soil Ecology*, 13(1), 45-55.

- Weltzin, J. F., Loik, M. E., Schwinning, S., Williams, D. G., Fay, P. A., Haddad, B. M., et al. (2003). Assessing the response of terrestrial ecosystems to potential changes in precipitation. *Bioscience*, 53(10), 941-952.
- West, G. B., Brown, J. H., & Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science*, 276(5309), 122-126.
- West, G. B., Brown, J. H., & Enquist, B. J. (1999). The fourth dimension of life: Fractal geometry and allometric scaling of organisms. *Science*, 284(5420), 1677-1679.
- Whitford, W. G., Freckman, D. W., Elkins, N. Z., Parker, L. W., Parmalee, R., Phillips, J., et al. (1981). Diurnal migration and responses to simulated rainfall in desert soil microarthropods and nematodes. *Soil Biology & Biochemistry*, 13(5), 417-425.
- Wood, F. H. (1973). Feeding relationships of soil-dwelling nematodes. *Soil Biol. Biochem.*, 5, 593-601.
- Wright, D. J. (1975). Elimination of nitrogenous compounds by *Panagrellus redivivus*, Goodey, 1945 (Nematoda: Cephalobidae). *Comparative Biochemistry and Physiology. B, Comparative biochemistry*, 52B, 247-253.
- Wright, D. J. (1975). Studies on nitrogen catabolism in *Panagrellus redivivus*, Goodey, 1945 (Nematoda: Cephalobidae). *Comparative Biochemistry and Physiology. B, Comparative biochemistry*, 52B, 255-260.
- Wright, P. A. (1995). Nitrogen excretion: Three end products, many physiological roles. *Journal of Experimental Biology*, 198(2), 273-281.
- Yeates, G. W., Bongers, T., de Goede, R. G. M., Freckman, D. W., & Georgieva, S. S. (1993). Feeding habits in soil nematode families and genera - an outline for soil ecologists. *Journal of Nematology*, 25(3), 315-331.
- Zheng, D. W., Agren, G. I., & Bengtsson, J. (1999). How do soil organisms affect total organic nitrogen storage and substrate nitrogen to carbon ratio in soils? A theoretical analysis. *Oikos*, 86(3), 430 to 442.

Figure 1. Growth Rate of Desert Protozoa.

Instantaneous growth rate (day^{-1}) of desert amoebae (squares), flagellates (triangles), and ciliates (circles) grown in liquid media, redrawn from Darby et al. (2006). Values below zero (negative growth) represent both encystment and mortality.

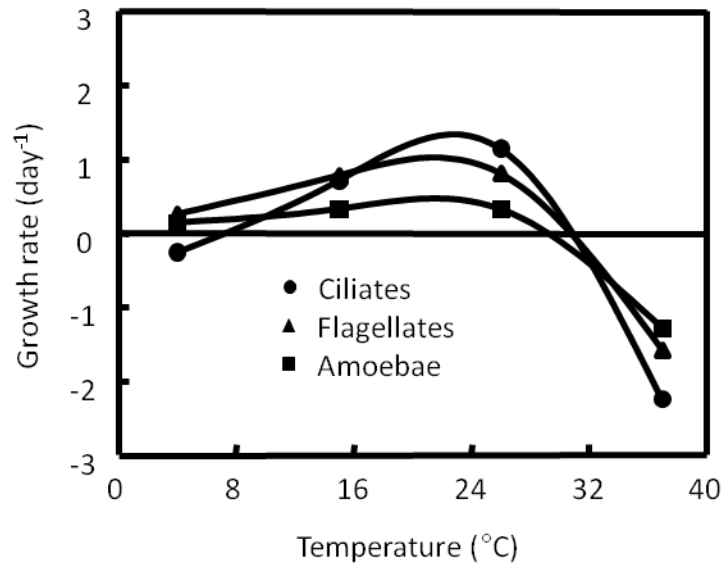


Figure 2. Desert Protozoa in Elevated Temperature and Precipitation.

Values represent mean biomass (mg kg^{-1}) of (A) amoebae, (B) nematodes, (C) flagellates, and (D) ciliates (\pm SE, $n = 30$) from mesocosm translocation experiment (Darby et al., 2006). Intact soil mesocosms were either left at the source location (solid bars) or moved to a destination location (shaded bars), intended to be warmer throughout the summer. All fauna experienced severe mortality when moved from the cool desert Colorado Plateau (COL) to the hot desert Chihuahuan Desert (CHI), but remained equal or increased in abundance when moved from the hot desert (CHI) to the even hotter Sonoran Desert (SON). Note the y-axis is represented in a \log_{10} to scale.

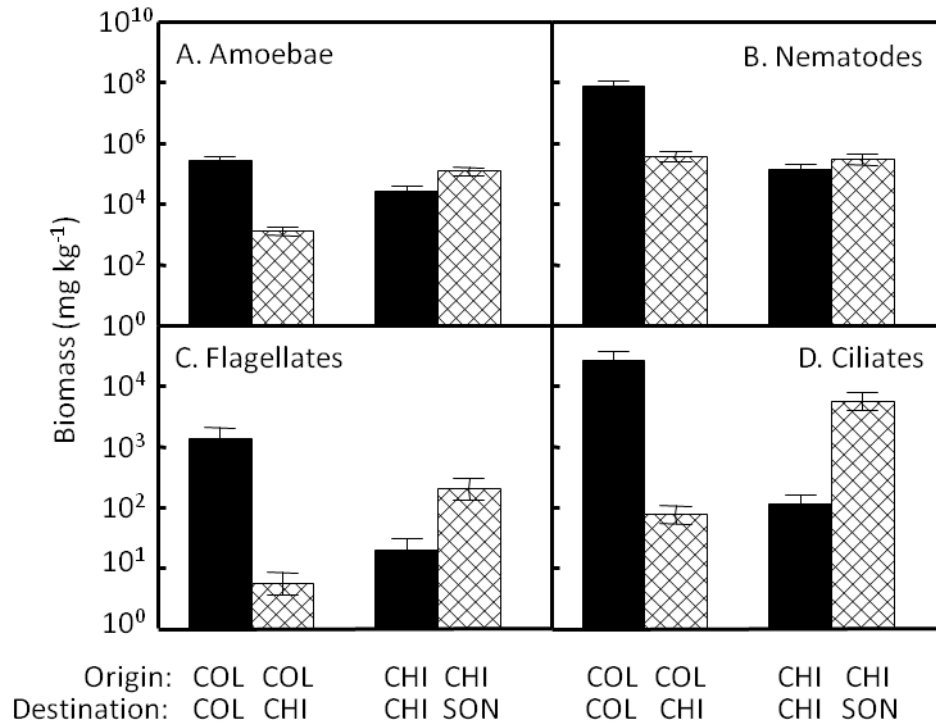


Figure 3. Conceptual Models of Nutrient Cycling in Fauna.

Top: physiological understanding that depicts production and mineralization as flowing from assimilated and biomass pools, respectively. Bottom: conceptual simplification from Hunt et al. (1987), depicting assimilation and production efficiency as a proportion of ingestion and assimilation, respectively.

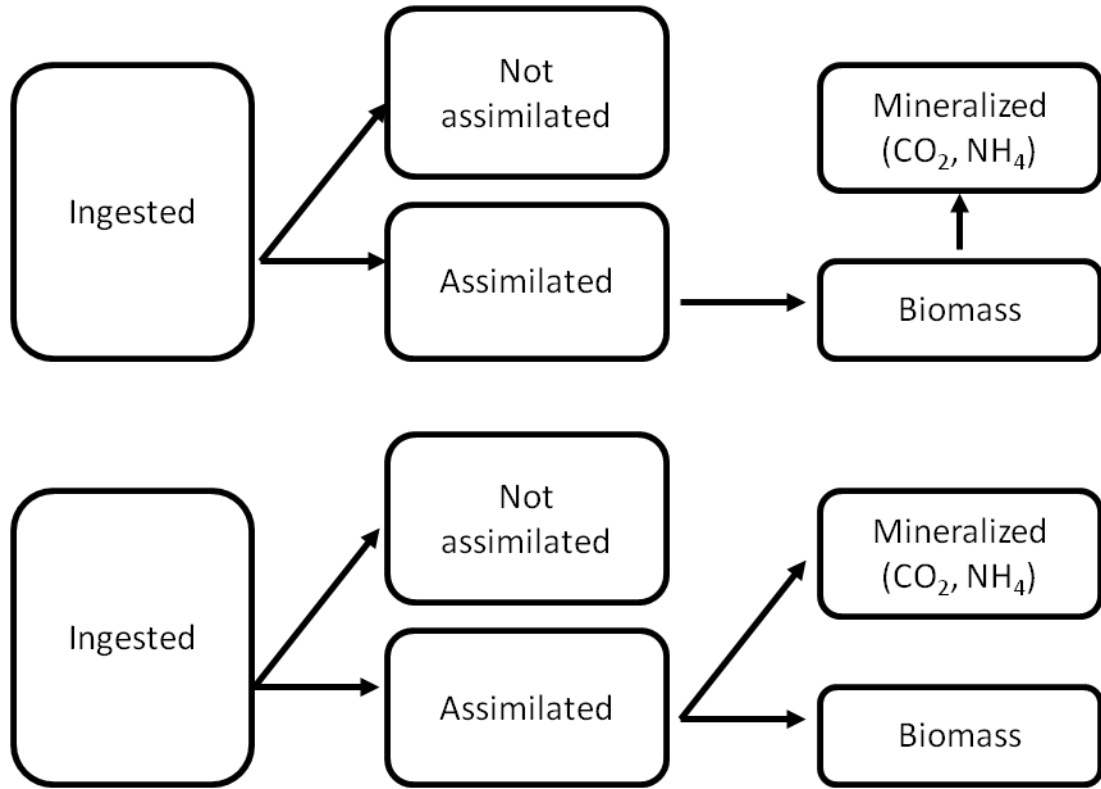
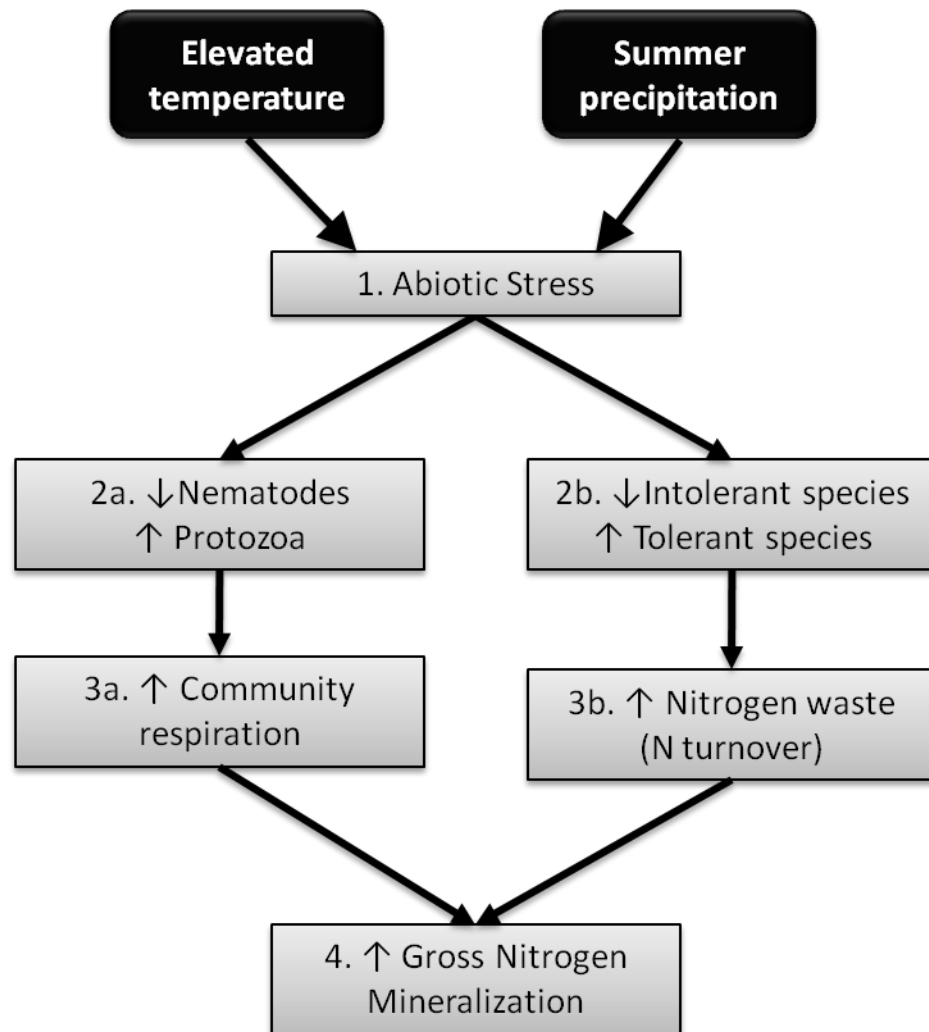


Figure 4. Hypothesized Consequence of Climate Change on Soil Microfauna.

1. Elevated temperature and summer precipitation is an abiotic stress for most soil microfauna. 2. Abiotic stress will affect (a) nematodes more negatively than amoebae, and (b) stress intolerant species more negatively than stress tolerant species. 3. If (a) protozoa respire more per unit of biomass than nematodes and (b) stress tolerant species accelerate nitrogenous wastes, then the net result will be (4.) greater relative contributions to gross nitrogen mineralization.



CHAPTER 2. GENERAL METHODOLOGY

This research relies heavily on the enumeration of nematodes and protozoa from soil, an opaque, heterogeneous habitat that impedes direct observation of inhabiting biota. A few prominent methods have emerged for extracting microfauna from soil, but no single method is ideal for every soil and application. The objective of this chapter is twofold, 1) document the methods used to enumerate nematode and protozoan communities in this dissertation, and 2) illustrate the various circumstances for which other methods would be better suited.

Foissner (1999) and Smirnov and Brown (2004) provide thorough reviews of the methods used for protozoa, but with differing perspective on the relative merits of each method. In short, the approaches for protozoa include some combination of two general principles: direct observation or indirect culturing. Direct observation might include diluting soil in a homogenous water slurry and counting all visible cells in aliquots of known volume on glass microslides and observed under 200x to 1000x magnification. Depending on the abundance and habit of the protozoan cells, this soil slurry may be too dense, or the protozoan cells too few, to count accurately or sufficiently distinguish from mineral soil particles. Alternatively, workers like Singh (1946) and Darbyshire et al. (1974) modified the most probable number method often used for counting bacteria in liquid cultures. Soil solution is diluted in a series and aliquots of the series are inoculated in wells of tissue culture plates or agar droplets in a petri dish and allowed to grow for 1 to 4 weeks. Each aliquot of each dilution level is inspected through time and scored for presence or absence of protozoa. The number of aliquots, and the dilution at which

aliquots are found to no longer contain viable cells, was used to estimate the ‘most probable number’ of cells according to the algorithm of Cochran (1950). An approach that combines direct observation and indirect culturing is the non-flooded petri dish method where a small amount of soil (3 to 5 g) is placed in a petri dish, sometimes on agar, and wetted with sterile water. Small aliquots are withdrawn periodically and assayed for the presence or absence of protozoan species.

Viglierchio and Schmitt (1983a, b) and Viglierchio and Yamashita (1983) offer a series of studies that review the methods used to separate nematodes from soil that represent extremes of a gradient, i.e., active movement of the nematode through a permeable membrane (passive on the part of the operator), or passive separation by density (active on the part of the operator). A Baermann funnel consists of soil placed on top of a filter paper in a funnel that is filled with water but stoppered from below. Nematodes are allowed to crawl through the soil and filter paper, into the water column below, and are collected 1 to 2 days later. Variations include placing the filter paper on a tray instead of funnel for greater surface area (also called Baermann trays or Whitehead trays), or first separating nematodes from most of the mineral soil by sieving or elutriation. Alternatively, nematodes are separated from mineral soil by density centrifugation using a salt or, more commonly, sucrose solution. To illustrate, the specific gravity of water is near 1.0 g cm^{-3} , 30 % sucrose is near 1.2 g cm^{-3} , most nematodes between 1.05 and 1.1 g cm^{-3} , and quartz near 2.6 g cm^{-3} . Therefore, mineral soil particles will sink in 30% sucrose while most nematodes will float and can be decanted or otherwise separated. A common criticism of the Baermann funnel and tray method is that

it requires the active movement of nematodes through the filter. Some plant-parasites or littoral bacterivores (e.g., Bunonematidae) are less mobile than others and will crawl through the filter paper of a Baermann funnel with less efficiency than other nematodes. A potential caveat of sucrose flotation is that detritus, fungal hyphae, and many other non-nematode particulates are co-separated with nematodes that leave a sample contaminated with debris.

Methods

Protozoa Extraction

Protozoa are enumerated from soils using a most probable number (MPN) technique from Darbyshire et al. (1974) with the following modifications (Darby et al., 2006). Sterilized soil extract (6% w/v) is prepared from each samples' native soil and used as the diluent for MPN dilutions. Nine grams of the original soil sample are mixed in 80 ml of sterile DI water and agitated on a rotary shaker for 5 min at room temperature. Five milliliters of this dilution is diluted 3-fold, seven times (in serial dilutions), and homogenous 1-ml aliquots of each of the final 6 dilutions are placed in each of eight wells across a 48-well Falcon[®] tissue culture plate row, one dilution per row. Wells positive for protozoa are recorded for each plate after 3, 10, 17, and 24 d of incubation at room temperature using an inverted microscope with DIC contrast at 100x, 200x, and 400x magnification. Repeated observations are necessary to observe the natural ecological succession in protozoan communities that develop in culture. Approximately 1 minute per well per week is spent seeking each motility group throughout the entire well, resulting in a search effort of about 30 to 60 min per plate per

week. The most probable number of each motility group (amoebae, flagellates, and ciliates) is calculated according to Cochran (1950). The minimum detection limit for this dilution series is estimated to be 7 cells g⁻¹ dry soil by calculating a hypothetical most probable number from a standard 3-fold dilution series initiated by 9.0 g soil. A minimum threshold is defined as a single positive well at the most concentrated dilution. Detection limits can be adjusted by including more or fewer dilution levels depending on the anticipated abundance. A subsample of the original soil is oven dried at 105 °C (48 h); water content [(fresh soil – dry soil) / dry soil] and protozoan abundance [(most probable number / fresh sample) * (fresh soil/dry soil)] are expressed on a dry weight basis. Abundance of each protozoan group is expressed on a dry mass basis, similar to nematodes.

Nematode Extraction

Cotton Filter Tray. Nematodes are extracted from soil with Cobb's decanting and sieving followed by Whitehead and Hemming cotton filter tray (Whitehead and Hemming, 1965). For sieving, soil is saturated and gently stirred into excess water (usually with 2 to 3 times the volume of soil) and decanted within 30 s of stirring (to allow the heaviest particles to settle but not the nematodes) with duplicate passes over 600, 250, 150, 75, and 44 µm sieves. The nematodes and organic debris caught on the sieves are backwashed into a common basin and poured onto a cotton filter suspended above a collecting tray. After 48 h, the cotton filter is removed and the nematodes that have actively crawled through the cotton filter are collected, settled, and condensed into 100 mL. All nematodes (and any tardigrades or rotifers also collected) are counted from

10 mL of a well-mixed 100 mL solution and assumed to be 10 % of the initial sample. Abundance of nematodes is expressed on a dry mass basis, similar to protozoa. Nematodes from the original sample are settled and condensed into a 15 mL conical tube and the addition of warm 8% formalin to 2 mL of the condensed nematode sample simultaneously heat relaxes and preserves the nematodes. Depending on the samples' concentration of nematodes and debris, four to six microslides are made from small aliquots of concentrated nematodes onto slides prepared with a paraffin wax ring that secures a cover slip when melted gently and cooled. Slides are painted with a thick ring of Glyptal 1201 ® red enamel to retain moisture. A uniform number of nematodes from these slides, generally 200 per sample depending on availability, are identified to genus according to Thorne (1974a, b), Jairajpuri and Ahmad (1992), Hunt (1993), Bongers (1994), Siddiqi (2000), and De Ley et al. (2003). Voucher specimens of observed genera, and species when possible, are created by slow-evaporation of formalin-preserved nematodes in dilute glycerin solutions (initially 5% glycerin, increasing to 100% at a rate of 5% per day) and mounted in anhydrous glycerin. A collection of the semi-permanent voucher specimens to date are stored in the D.A. Neher lab in the Department of Plant and Soil Science, University of Vermont, Burlington, VT.

Sucrose Flotation. An alternative approach to extract nematodes from the mineral soil is through density separation with concentrated sucrose solution. A variety of approaches have been developed from a number of research groups using a number of different soils (Freckman et al., 1977, Jenkins 1964). The method used here begins with Cobb's sieving (as above) over a similar series of sieves, backwashing and collecting

material caught on sieves into a common container that is eventually condensed, by settling, siphoning, and transferring to increasingly smaller containers, ultimately into a 50 to ml centrifuge tube. The nematodes in water are centrifuged and the supernatant siphoned to leave 5 ml. The centrifuge tube is filled with sucrose solution (1.5 mol L^{-1} , or approximately 50% v/v) to a final concentration of approximately 1.33 mol L^{-1} (final specific gravity of approximately 1.17 g cm^{-3}) and centrifuged for 2 min. The supernatant containing the floating nematodes was quickly decanted into a jar half-filled with water so as to provide immediate relief from the negative osmotic balance of the sucrose solution. Nematodes were passed over a $25 \mu\text{m}$ sieve, washed of residual sucrose with a gentle drizzle of water, and backwashed back into a clean container for counting and preservation as described above.

Methods Comparison. To compare the extraction efficiency of the sieving and cotton filter technique with the sucrose flotation technique, 25 samples collected at the conclusion of the field experiment (5 treatments from each of 5 blocks, details in chapter 5) were homogenized gently and split into two parts. Nematodes were extracted from 200 g through the sieving and cotton filter technique (described above) and from 100 g through the sucrose flotation technique. Nematodes were counted and corrected for sample size and moisture content. Previous work showed that gentle homogenization of relatively dry soil results in comparable subsamples making a simple correlation of paired subsamples reasonable. Data were analyzed by Pearson correlation to assess extraction fidelity and by linear regression (intercept set to zero) to estimate extraction efficiency as

the slope coefficient. Correlation and linear regression was performed with the CORR and MIXED procedures, respectively, of SAS software (SAS Institute, Inc., Cary, NC).

Auxiliary Experiments

A variety of experiments were performed to illustrate objectives for which an alternative method of extracting nematodes would be used.

Density. To estimate the specific density of the desert nematodes, three large (1 kg) samples were collected in fall 2007. Nematodes were extracted from three equal subsamples and later recombined to result in approximately 4,000 individuals per sample. Each sample was stirred and poured equally into 12 test tubes. The nematodes in each test tube were centrifuged and the water siphoned down to 0.5 ml. Sucrose solutions were prepared with high-purity water and granulated cane sugar (oven dried to equilibrium at 65 °C to remove water) in a density gradient with 11, 13, 15, 17, 19, 20, 21, 23, 25, 27, 29, and 31 °Brix. °Brix units represent the mass ratio of sucrose dissolved to 100 ml, so 25 °Brix represents 25 g sucrose dissolved to 100 ml. Each of the 12 test tubes per sample were filled rapidly, to homogenize, to 14 ml with one of the 12 sucrose solutions and centrifuged for 4 min. The top half of the supernatant was poured into a separate container and both the top and bottom portions were immediately diluted with distilled water to relieve the nematodes of the osmotic stress from higher molarity sucrose solutions. The density of the sucrose solutions used was estimated from the final molarity of the solution (corrected for the 0.5 ml water left in the test tube) according to the data presented in Lide (2001-2002). Nematodes collected from the top were assumed to be lighter than the solution, nematodes collected from the bottom were assumed to be

heavier than the solution, and the proportion of nematodes floating was modeled by logistic regression using the LOGISTIC procedure of SAS software.

Depth. Anhydrobiotic nematodes assume a coiled habitus when dry but gradually uncoil during rehydration following a wetting event. Freckman et al. (1977) illustrated the utility of the sucrose flotation technique to extract both hydrated and coiled nematodes. To illustrate how anhydrobiosis is as relevant to soil functioning as total abundance, the proportion of the community that is anhydrobiotic was compared to that at depth. Soil (100g, 0 to 20 cm) was collected from five plots in September 2005 and 0 to 10 cm soil was kept separate from 10 to 20 cm soil. For each of five plots, 50 g soil from the surface 0 to 10 cm was added directly to a test tube and filled with 6% formalin within 1 min from the time of sampling and 50 g deep soil (10 to 20 cm) was also added to a test tube and filled with 6% formalin. Nematodes were extracted from all 10 samples by sucrose flotation and the resulting nematodes were counted and picked individually onto glass microslides and enumerated to genus and scored as being active (uncoiled) or anhydrobiotic (coiled). Coiled nematodes were defined as those whose tail tip coiled to reach at least past the plane of the head. Nematode abundance and gravimetric soil moisture at the time of sampling were compared between depths by paired *t*-tests using the MIXED procedure and the proportion of individuals coiled and uncoiled for each genus was compared between depths by chi-square tests using the FREQ procedure of SAS software.

Rehydration. To test the rate at which nematodes uncoil following a rain event, three large (1kg) samples were collected and divided into eleven 20 to g subsamples in

sealed test tubes. Each subsample was re-wetted with water to near holding capacity and later saturated with 10% formalin (to a final concentration of 8%) at 0, 5, 10, 15, 20, 30, 40, 50, 60, 90, and 120 min. Nematodes were extracted from each subsample by sucrose flotation, enumerated to genus, and scored as coiled or uncoiled, which was arrested at the addition of formalin. The proportion of nematodes uncoiled was modeled with logistic regression using the LOGISTIC procedure of SAS software for three groups of nematodes: 1) Tylenchidae, 2) Cephalobidae, and 3) all nematodes together.

Results

Methods Comparison. Nematode abundance was correlated positively between cotton-filter and sucrose extraction methods ($R^2 = 0.6792$, forced through origin) with a slope greater than one ($\beta = 1.28$, $p < 0.0001$, Figure 5), indicating that the extraction efficiency of the cotton filter technique was approximately 128% that of sucrose flotation for these communities.

Density. Nematode recovery from the surface of the sucrose solutions followed the logistic model (Figure 6), and the parameters fit to the logistic function estimate that the median nematode density was 1.0668 g cm^{-3} . Only data from the sucrose solutions with densities less than 1.10 g/cm^3 were used because recovery from greater densities appeared to be susceptible to an artifact of hydrophobicity or extreme osmotic imbalance (reviewed in Carta and Carta 2000).

Depth. Nematodes were more abundant at the surface (0 to 10 cm) than at depth (10 to 20 cm) ($p = 0.0097$), but a greater portion of the community was uncoiled (and presumed active at the time of sampling) at depth than at the surface. More total active

nematodes were at depth than at the surface (Figure 7). Gravimetric moisture at depth (2.97 %) was nearly three times greater than at the surface (1.08 %) ($p = 0.0022$).

Although most genera were more frequently coiled at the surface than at depth (Table 1), some genera, like *Tylenchus*, *Chiloplacus*, *Drilocephalobus*, *Acromoldavicus*, *Ecumenicus*, and *Aporcelaimellus*, did not differ in coiling between the surface and at depth.

Rehydration. Uncoiling of nematodes following re-hydration followed the logistic model (Figure 8), and the parameters fit to the logistic function estimate that half the nematodes will be uncoiled after 14.4 min. Tylenchidae (mostly the genus *Tylenchus*) and Cephalobidae were the only taxa with enough individuals represented to estimate taxa-specific parameters with statistical confidence. The rate at which Tylenchidae uncoil was about twice as fast as Cephalobidae (Table 2).

Discussion

Protozoa Methods

The methods used by primary research of desert protozoan depend on the objective of the study. When the objective is to detect the species present in a sample (Bamforth, 1984, 2004; Robinson et al., 2002) a direct observation approach, such as a flooded petri dish or individual fresh-mount microslides prepared from soil slurry, is recommended. When the objective calls for an estimate of total abundance (Whitford et al., 1981; Parker et al., 1984; Whitford et al., 1989; Rodrigues-Zaragoza and Steinberger, 2004; Rodriguez-Zaragoza et al., 2005; Belnap et al., 2001, 2005; Darby et al., 2006), indirect culturing methods, such as the most probable number approach, are

recommended. This is for two practical reasons. First, although direct counts may require less time per sample in the long run, the counting must be performed soon after sampling for the data to be representative of the community at the time of sampling. If each sample were to take one hour with an operator enumerating ten samples per day, the communities may easily change within the five days it takes to process an experiment of 50 samples. The most probable number culturing approach allows the operator to spend less time per sample immediately after sampling; more time is spent per sample but the labor is extended over 3-4 weeks. Second, many protozoan cells forage in or around soil particles, clumps, or micro-aggregates and are undetectable under direct observation. Indirect culturing methods do not necessarily require that all enumerated cells be in continuous culture, but rather that all enumerated cells be visible at least once during the 4-week observations. This less-stringent criterion reflects the notion that the most probable number approach is more representative of the small, 'shy' cells that make up the bulk of the abundance.

Nematode Methods

Recovery of desert nematodes from the sieving and cotton filter technique was comparable to and slightly greater than recovery from sucrose flotation, a finding that may be unique to desert nematodes. Past experience with nematode extractions suggests that resident soil, litter, or other debris on top of the filter paper contributes to variability and the loss of recovery efficiency. Sieved nematodes on a cotton filter with little other debris seem more prone to crawl through the filter than non-sieved nematodes surrounded by bulk soil. This means that past comparisons of nematode extraction techniques may

overestimate the benefit of the sucrose flotation technique when comparing sucrose flotation to Baermann funnels or trays (bulk soil placed on suspended filters) without prior separation or sieving of the nematodes from surrounding soil aggregates.

The specific gravity (g cm^{-3}) of nematodes extracted from this desert community matches a number of previous estimates (Carta and Carta, 2002, Table 3), but it just less than the specific gravity used by a majority of studies that estimate total community biomass based on length and width measurements (Andrássy 1956). The nematodes of this study were fully hydrated at the time of density centrifugation, but may have egested some intestinal contents. More work is needed to determine whether the discrepancy in estimates of specific gravity is an artifact of methodological differences or a result of nematode community composition or nutritional state. Precise estimates of specific gravity could lead to more accurate estimates of community biomass.

Although not used in routine nematode community analysis, sucrose flotation was an effective way to extract anhydrobiotic nematodes from soil if preserved *in situ* as a way to determine whether nematodes were active or anhydrobiotic at the time of sampling (Freckman et al., 1977; Treonis et al., 2000). During a typical warm late summer day, it is possible for there to be more actively grazing nematodes at deeper soils (10 to 20cm) than at the surface (0 to 10 cm), even though total abundance is greater at the surface. Many of the dorylaim-type predator/omnivore nematodes in the Colorado Plateau (e.g., *Discolaimium*, *Eudorylaimus*, *Aporcelaimellus*) are a greater portion of the community at 10 to 20 cm than at 0 to 10 cm (Darby et al., 2007). I hypothesize that deep soils (>10 cm) may retain more moisture in vapor form than surface soils (< 10 cm) and

allow a greater portion of these relatively long-lived, slower growing species to be active for a longer period of time. In contrast, the nematodes at the surface (0 to 10 cm) are characteristic of short-lived, fast-growing species that are more tolerant of drought and desiccation. For example, the proportion of individuals coiled for *Tylenchus* and *Acromoldavicus*, two of the three most abundant genera, was not greater significantly at the surface (0 to 10 cm) than at depth (10 to 20 cm) even though deeper soil had approximately three times the moisture content. After a wetting event, it takes less than 15 min for most nematodes to appear uncoiled, and at least 95 % of the community is uncoiled after 60 min. However, the physiological demands of resuming activity elevates metabolism during rehydration, and Crowe et al., (1977) showed that metabolism does not return to normal basal rates until 1-2 h after re-hydration. Thus, several hours to days, depending on the individual species, may be needed for nematodes to consume enough prey to match the energetic demands of anhydrobiosis (Madin et al., 1985).

References

- Andrássy, I. (1956). Die rauminhalts- und gewichtsbestimmung der fadenwürmer (Nematoden). *Acta Zoologica Hungarica*, 2, 1-15.
- Bamforth, S. S. (1984). Microbial distributions in Arizona deserts and woodlands. *Soil Biology & Biochemistry*, 16(2), 133-137.
- Bamforth, S. S. (2004). Water film fauna of microbiotic crusts of a warm desert. *Journal of Arid Environments*, 56(3), 413-423.
- Belnap, J., & Phillips, S. L. (2001). Soil biota in an ungrazed grassland: Response to annual grass (*Bromus tectorum*) invasion. *Ecological Applications*, 11(5), 1261-1275.
- Belnap, J., Phillips, S. L., Sherrod, S. K., & Moldenke, A. (2005). Soil biota can change after exotic plant invasion: Does this affect ecosystem processes? *Ecology*, 86(11), 3007-3017.
- Bongers, T. (1994). *De Nematoden van Nederland*. Utrecht, The Netherlands: Koninklijke Nedelandse Natuuhistorische Vereniging.
- Carta, L. K., & Carta, D. G. (2000). Nematode specific gravity profiles and applications to flotation extraction and taxonomy. *Nematology*, 2, 201-210.

- Cochran, W. G. (1950). Estimation of bacterial densities by means of the "most probable number". *Biometrics*, 6, 105-116.
- Crowe, J. H., Madin, K. A. C., & Loomis, S. H. (1977). Anhydrobiosis in nematodes: Metabolism during resumption of activity. *Journal of Experimental Zoology*, 201, 57-64.
- Darby, B. J., Housman, D. C., Zaki, A. M., Shamout, Y., Adl, S. M., Belnap, J., et al. (2006). Effects of altered temperature and precipitation on desert protozoa associated with biological soil crusts. *Journal of Eukaryotic Microbiology*, 53(6), 507-514.
- Darby, B. J., Neher, D. A., & Belnap, J. (2007). Soil nematode communities are ecologically more mature beneath late- than early-successional stage biological soil crusts. *Applied Soil Ecology*, 35(1), 203-212.
- Darbyshire, J. F., Wheatley, R. E., Greaves, M. P., & Inkson, R. H. E. (1974). A rapid micromethod for estimating bacteria and protozoan populations in soil. *Revue D Ecologie Et De Biologie Du Sol*, 11, 465-475.
- De Ley, P., De Ley, I. T., Mundo-Ocampo, M., Mundo, L., & Baldwin, J. (2003). *Identification of freeliving nematodes (secernentea)*. Riverside, California: University of California Extension.
- Foissner, W. (1999). Soil protozoa as bioindicators: Pros and cons, methods, diversity, representative examples. *Agriculture Ecosystems & Environment*, 74(1-3), 95-112.
- Freckman, D. W., Kaplan, D. T., & van Gundy, S. D. (1977). A comparison of techniques for extraction and study of anhydrobiotic nematodes from dry soils. *Journal of Nematology*, 9(2), 176-181.
- Hunt, D. J. (1993). Aphelenchida, Longidoridae and Trichodoridae: Their systematics and bionomics. Cambridge, UK: CAB International.
- Jairajpuri, M. S., & Ahmad, W. (1992). Dorylaimida: Free-living, predaceous and plant-parasitic nematodes. New York: E.J. Brill.
- Jenkins, W. R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter*, 48(9), 692.
- Lide, D. (2001-2002). Handbook for chemistry and physics, 82nd edition. New York: CRC Press.
- Madin, K. A. C., Loomis, S. H., & Crowe, J. H. (1985). Anhydrobiosis in nematodes - control of carbon flow through the glyoxylate cycle. *Journal of Experimental Zoology*, 234(3), 341-350.
- Parker, L. W., Freckman, D. W., Steinberger, Y., Driggers, L., & Whitford, W. G. (1984). Effects of simulated rainfall and litter quantities on desert soil biota - soil respiration, microflora, and protozoa. *Pedobiologia*, 27(3), 185-195.
- Robinson, B. S., Bamforth, S. S., & Dobson, P. J. (2002). Density and diversity of protozoa in some arid Australian soils. *Journal of Eukaryotic Microbiology*, 49(6), 449-453.
- Rodriguez-Zaragoza, S., Mayzlish, E., & Steinberger, Y. (2005). Vertical distribution of the free-living amoeba population in soil under desert shrubs in the Negev Desert, Israel. *Applied and Environmental Microbiology*, 71(4), 2053-2060.

- Siddiqi, M. R. (2000). *Tylenchida: Parasites of plants and insects*, 2nd ed. Cambridge, UK: CAB International.
- Singh, B. N. (1946). A method of estimating the numbers of soil protozoa, especially amoebae based on their differential feeding on bacteria. *Annals of Applied Biology*, 33, 112-119.
- Smirnov, A. V., & Brown, S. (2004). Guide to the methods of study and identification of soil gymnamoebae. *Protistology*, 3(3), 148-190.
- Thorne, G. (1974). *Nematodes of the Northern Great Plains. Part I. Tylenchida (Nemata: Secernentea)*. Brookings, SD: Tech. Bull. 41, Agricultural Experiment Station, South Dakota State University.
- Thorne, G. (1974). *Nematodes of the Northern Great Plains. Part II. Dorylaimoidea in part (Nemata: Adenophorea)*. Brookings, SD: Tech. Bull. 41, Agricultural Experiment Station, South Dakota State University.
- Treonis, A. M., Wall, D. H., & Virginia, R. A. (2000). The use of anhydrobiosis by soil nematodes in the Antarctic dry valleys. *Functional Ecology*, 14(4), 460 to 467.
- Viglierchio, D. R., & Schmitt, R. V. (1983). On the methodology of nematode extraction from field samples - Baermann funnel modifications. *Journal of Nematology*, 15(3), 438-444.
- Viglierchio, D. R., & Schmitt, R. V. (1983). On the methodology of nematode extraction from field samples - comparison of methods for soil extraction. *Journal of Nematology*, 15(3), 450 to 454.
- Viglierchio, D. R., & Yamashita, T. T. (1983). On the methodology of nematode extraction from field samples - density flotation techniques. *Journal of Nematology*, 15(3), 444-449.
- Whitehead, A. G., & Hemming, J. R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, 55, 25-38.
- Whitford, W. G., Aldon, E. F., Freckman, D. W., Steinberger, Y., & Parker, L. W. (1989). Effects of organic amendments on soil biota on a degraded rangeland. *Journal of Range Management*, 42(1), 56-60.
- Whitford, W. G., Freckman, D. W., Elkins, N. Z., Parker, L. W., Parmalee, R., Phillips, J., et al. (1981). Diurnal migration and responses to simulated rainfall in desert soil microarthropods and nematodes. *Soil Biology & Biochemistry*, 13(5), 417-425.
- Zaragoza, S. R., & Steinberger, Y. (2004). Seasonal dynamics of amoebae in the root canopy of *Zygophyllum dumosum* in the Negev Desert, Israel. *Pedobiologia*, 48(3), 277-281.

Table 1. Nematode Activity by Depth.

Nematode activity (number active / total) found at 0 to 10 cm and 10 to 20 cm soils with χ^2 tests (df = 1) for homogeneity between depths within a genus. Data represent all individuals collected from five 40 to cm³ samples per depth but originating from five separate plots.

<u>Genus</u>	<u>0 to 10 cm</u>	<u>10 to 20 cm</u>	<u>χ^2</u>
<i>Acrobeles</i>	4/181	9/51	17.93***
<i>Acrobeloides</i>	0/16	0/0	.
<i>Acromoldavicus</i>	2/311	0/39	0.25
<i>Aphelenchoides</i>	0/3	1/2	4.80*
<i>Aphelenchus</i>	0/0	3/3	.
<i>Aporcelaimellus</i>	2/5	6/10	0.54
<i>Axonchium</i>	0/0	1/1	.
<i>Cephalobus</i>	0/0	0/1	.
<i>Cervidellus</i>	0/16	4/6	13.04***
<i>Chiloplacus</i>	1/1	6/7	0.16
<i>Discolaimium</i>	0/18	22/28	27.11***
<i>Drilocephalobus</i>	0/2	1/1	3.00
<i>Ecumenicus</i>	1/2	0/1	0.75
<i>Eudorylaimus</i>	0/9	1/1	10.00**
<i>Filenchus</i>	0/0	2/3	.
<i>Kochinema</i>	0/0	2/2	.
<i>Nothacrobeles</i>	0/0	0/1	.
<i>Nygolaimus</i>	0/3	5/5	8.00**
<i>Placodira</i>	0/1	0/0	.
<i>Plectus</i>	0/2	0/0	.
<i>Tylenchorhynchus</i>	0/0	3/3	.
<i>Tylenchus</i>	8/61	1/10	0.08
<i>Xiphinema</i>	0/0	1/1	.
Total	18/631	68/176	185.08***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2. Nematode Rehydration after Wetting Event.

Logistic regression parameters (with standard error) of total nematodes uncoiling after a rehydrating event, including two families (Tylenchidae and Cephalobidae) frequent enough to provide separate analysis. Parameters of the logit function ($\ln(p/1-p)$, where p = probability of floating) were fit to the equation $\text{logit}(p) = \beta * x + \alpha$ (where x = time since wetting). The mean time at which 50 % of the individuals uncoil (ET_{50}) is estimated as $(-\alpha / \beta)$.

<u>Taxa</u>	<u>Intercept (α)</u>	<u>Slope (β)</u>	<u>ET_{50} (min)</u>
All	-1.1530 (0.1824)	0.0801 (0.0078)	14.4
Tylenchidae	-2.3379 (0.7273)	0.1984 (0.0517)	11.8
Cephalobidae	-2.4382 (0.3270)	0.1016 (0.0118)	24.0

Table 3. Nematode Specific Gravity.

Nematode specific gravity estimates from a variety of different sources and methods.

<u>Study (+ species covered)</u>	<u>Approach</u>	<u>Density (g cm⁻³)</u>
Overgaard-Nielsen, 1949 <i>Mononchus papilatus</i>	salt gradient	1.02
Caveness and Jensen, 1955 <i>Pratylenchus penetrans</i> , <i>Rhabditis</i> sp., <i>Diplogaster</i> sp., <i>Longidorus sylphus</i>	sucrose	1.05-1.06
Andrássy , 1956 unspecified	salt and acids	1.082-1.086
Thistlethwayte and Riedel, 1969 unspecified	literature	1.04-1.09
Carta and Carta, 2000 <i>Pratylenchus penetrans</i> adults <i>Pratylenchus agilis</i> adults <i>Pratylenchus scribneri</i> adults <i>Caenorhabditis elegans</i> adults	sucrose	1.058 1.068 1.073 1.091
Darby (present study) whole community	sucrose	1.067

Figure 5. Nematode Extraction Efficiency.

Abundance of total nematodes extracted from sieving and cotton filter (vertical axis) compared with nematodes recovered from sucrose flotation (horizontal axis). Regression trendline (solid line) was forced through the origin (0,0) and is compared with the 1:1 line (dashed line).

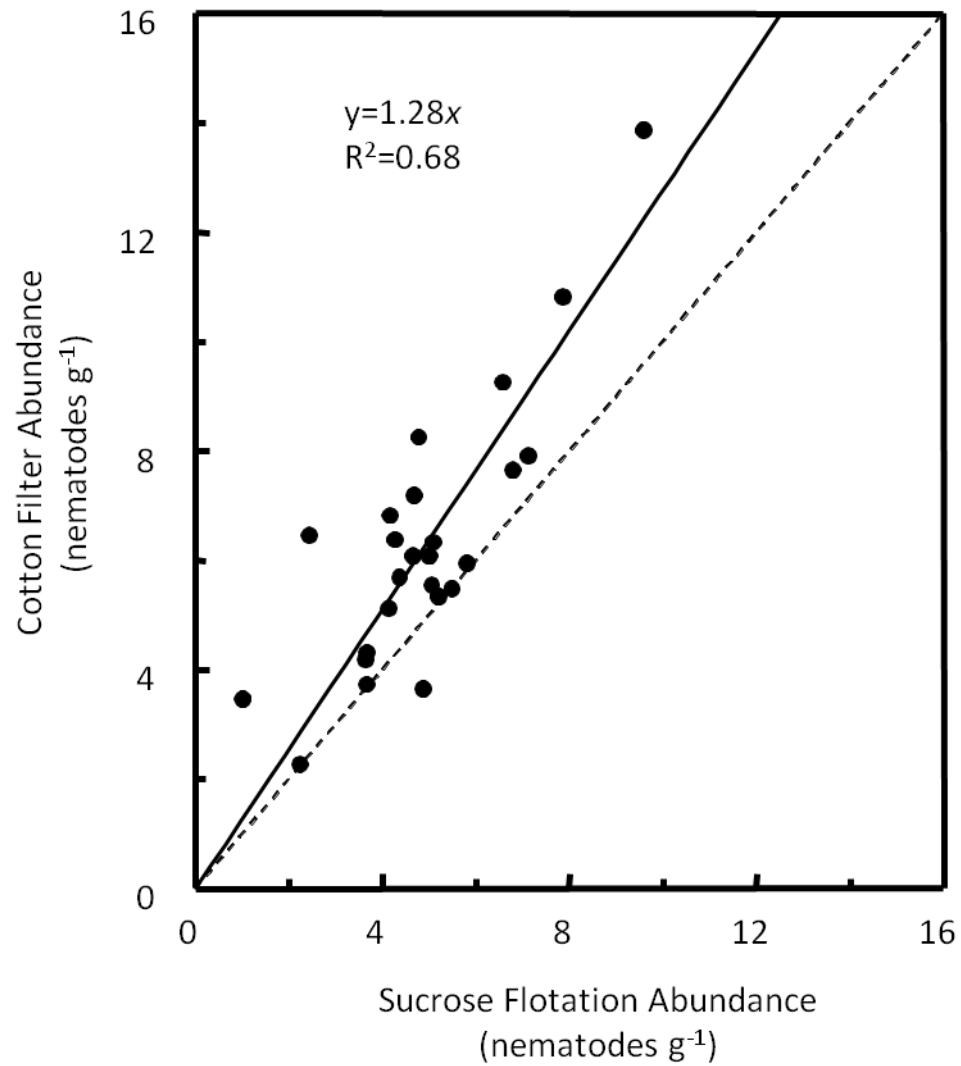


Figure 6. Nematode Specific Gravity.

Nematode recovery from a density gradient of sucrose solutions. The parameters of the logit function ($\log(p/1-p)$, where p = probability of floating) were fit to the equation $\text{logit}(p) = 93.2038x + -99.4277$ (solid line, where x = solution density in g cm^{-3}). The mean density (at which 50% of the individuals float) is estimated at 1.0668 g cm^{-3} (dashed lines). Values greater than 1.0 g cm^{-3} (empty circles) were not included as showing evidence of an artifact of hydrophobicity or extreme osmotic imbalance by concentrated sucrose.

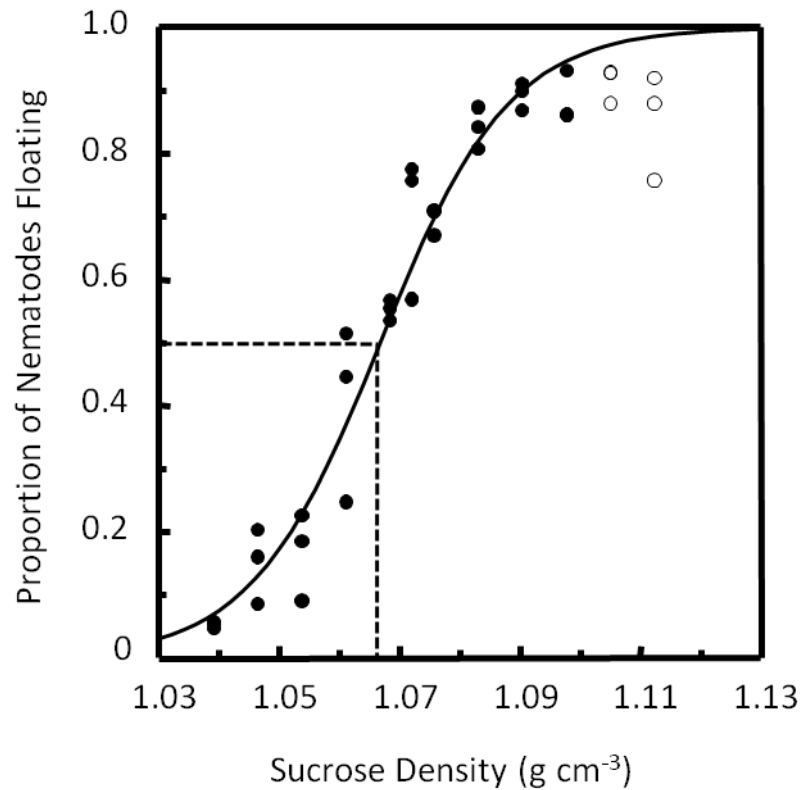


Figure 7. Nematode Activity by Depth.

Mean nematode abundance (\pm SE) at 0 to 10 cm and 10 to 20 cm depth illustrating the proportion of individuals uncoiled (i.e., active, dark portion of bars) and coiled (i.e., inactive, light portion of bars).

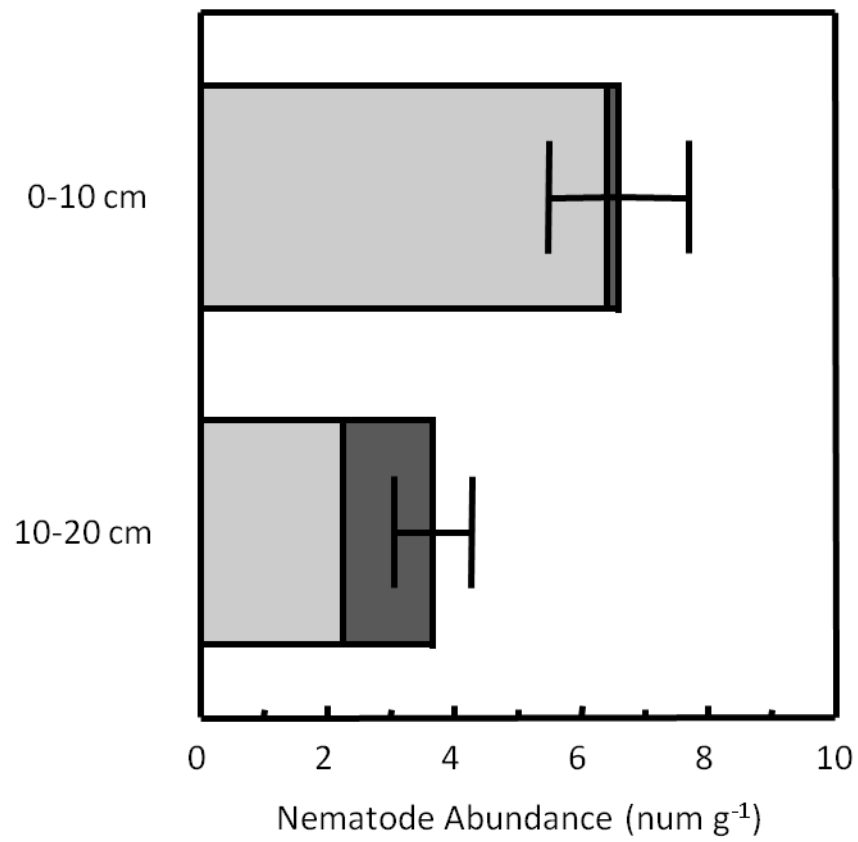
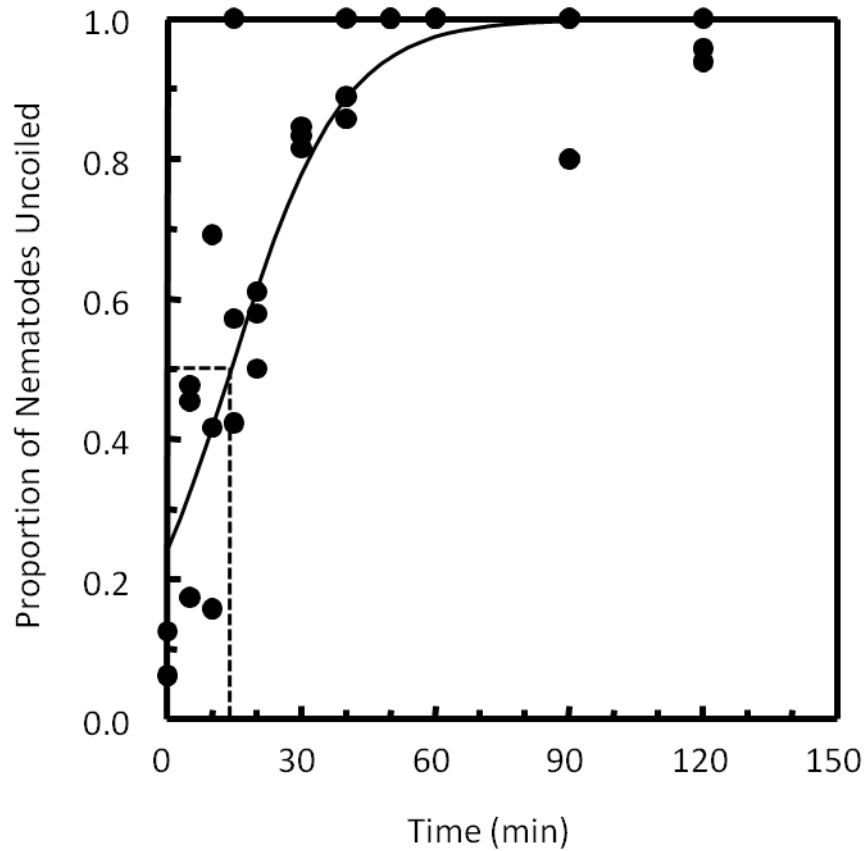


Figure 8. Nematode Rehydration after Wetting Event.

Proportion of nematodes uncoiling following a wetting event. Parameters of the logit function ($\log(p/1-p)$, where p = proportion of nematodes uncoiled, solid line) was fit to the equation $\text{logit}(p) = 0.0801x + -1.1530$ (where x = time since wetting). Time at which 50 % of the individuals were uncoiled is estimated at 14.4 min (dashed line).



CHAPTER 3. ENERGY PATHWAYS IN THE DESERT SOIL FOOD WEB

Carbon for the desert soil food web is supplied by vascular and non-vascular plants as well as photosynthetic cyanobacteria. A significant portion of nitrogen inputs to the food web is from biological fixation by nitrogen fixing cyanobacteria, lichens, and plant-associated bacteria in rhizospheres, mostly alpha-proteobacteria. Nutrients in desert soils are thought to concentrate near desert shrubs (Schlesinger et al., 1996). Shrubs relocate interspace soil nutrients to rhizosphere soil through litterfall and dust entrapment (Schlesinger and Pilmanis, 1998). Shrubs and invasive bunchgrasses often outcompete native grasses in nutrient-poor soils, leading to further heterogeneity of soil resources and desertification of marginal lands (Schlesinger et al., 1990). However, Housman et al. (2007) demonstrated that only some, but not all, nutrients are concentrated consistently near plants.

In contrast to vascular plants that sometimes form islands of fertility, biological soil crusts are a distinctive feature of desert soils that represents up to 70% of the living soil cover and can represent more of a carpet of fertility. Early-colonizing fungi and cyanobacteria stabilize the soil surface and facilitate colonization by lichens, green algae, and mosses. Biological soil crusts increase the physical stability of surfaces and soil fertility through dust entrapment, photosynthesis, nitrogen fixation, and mineral chelation (reviewed in Belnap, 2003). The autotrophic components of crusts are sensitive to surface

disturbances and climate changes, such as altered temperature and precipitation (Belnap, 2003). For example, rapid wetting and drying cycles reduce quantum yield, chlorophyll, and ultraviolet protective pigments of soil lichens (Belnap et al., 2004), and high temperatures ($>26^{\circ}\text{C}$) inhibit nitrogen fixation (Belnap, 2002). Biological soil crusts mediate some environmental stresses by fixing carbon (Beymer and Klopatek, 1991) and nitrogen (Rychert and Skujins, 1974; West and Skujins, 1977; Rychert et al., 1978; Jeffries et al., 1992), chelating dust-blown minerals (Reynolds et al., 2001), increasing moisture retention (George et al., 2003), and limiting losses to wind-erosion (Williams et al., 1995; Belnap and Gillette 1997, 1998; Hu et al., 2002). Biological soil crusts have an important influence on ecosystem functioning and the soil food web (Evans and Johansen, 1999; Belnap, 2003), but they are highly vulnerable to physical compaction disturbances including off-road vehicles and human recreation and hiking (Belnap, 1995, 2002; Belnap and Warren, 2002). Surface disturbances accelerate desertification (Belnap, 1995) by reducing biological crust cover and, thus, alter desert nutrient dynamics (Evans and Belnap, 1999).

The objective of this chapter is to introduce the desert soil water film fauna by examining the dynamics of the main trophic links and energy pathways in the desert soil food web through two main experiments. First, the Plant Experiment compares nematode genera associated with the plant rhizosphere with interspace communities. These experiments are part of a larger experiment, part of which has been published previously (Housman et al., 2007). Nematodes were found to be more abundant near plants (Figure

9), but neither the abundance of any protozoan group, nor the relative proportion of nematode trophic groups, was affected by distance from plants. The principle question for this chapter is to determine whether nematode genera increase in abundance uniformly from interspaces to the plant rhizosphere, or whether some genera increase disproportionately more or less than others. Secondly, the Crust Removal Experiment compares the bacterial decomposition pathway with the fungal pathway by examining the long-term effects of chronic physical disturbance that effectively removes the biological crust formation. Similar studies have shown that trampling of desert soils diminish the surface biota and result in bacteria taking a more prominent portion of the decomposition energy pathway than fungi (Belnap 1995). Similarly, long-term trampling of desert soils is hypothesized to result in a greater proportion of bacterivorous nematodes than fungivorous nematodes to reflect the shift in prey communities.

Methods

Plant Experiment

Soil was collected from two locations near Moab, Utah, in September 2003. The first location was just outside the Island in the Sky region of Canyonlands National Park (here referred to as “I-Sky”), and the second in the Needles section of the same park (here referred to as “Needles”). Canyonlands National Park represents a cool desert of the Colorado Plateau (Rosentreter and Belnap, 2001). Within each location, one area was identified that was dominated by the grass *Stipa hymenoides* and a second area dominated by the shrub *Coleogyne ramosissima*. In both plant type areas at both locations, portions

of the biological soil crust cover were identified as belonging to one of two categories of crust cover. The first category was defined as crust that is dominated by the cyanobacteria *Microcoleus vaginatus*, representing a relatively early successional stage, and here called “cyanobacteria crusts”. The second category of crust contains a more diverse assemblage of flora including the cyanobacteria *M. vaginatus*, *Scytonema myochrous*, and *Nostoc commune*, the lichen *Collema tenax* and *C. coccophorum*, and the mosses *Syntrichia* sp. and *Bryum* sp. These represent a relatively late-successional stage and are here called “cyano/lichen/moss crusts”. At both locations, six plots were identified within each plant area for a total of three replicate plots for each crust type. Each plot consisted of three plants of the same species and associated with the same crust type. Soil cores were collected from the top 0 to 10 cm in each of the four cardinal directions around each plant at five microsites: stem, dripline, close (3 cm), mid (10 cm), and far (35 cm) interspaces (measured from the edge of the canopy dripline). Cores from the same microsite were pooled from all three plants within the plot. Thus, the sampling design included 2 locations, 2 plant types, 2 crust types, 3 replicate plots of each location, plant and crust combination, and 5 microsites per plot. A mean of 250 representative individuals per experimental unit were identified to genus. All non-stylet bearing genera were considered a single bacterivorous trophic group.

Crust Removal Experiment

A second experiment sought to determine if biological soil crust removal, though a chronic, moderate level physical disturbance (i.e., trampling), affects the associated soil

nematode and protozoan communities. The sites were selected to span a range of profile depth, including a shallow profile (i.e., 10 cm to bedrock), a medium profile (i.e., 20 cm to bedrock), and a deep profile (i.e., 30 cm to bedrock). The shallow and medium profile are located in the Island in the Sky region of Canyonlands National Park and the deep profile is located in Arches National Park, both near Moab, Utah. At each profile, ten plots of 10 m² area were delineated and five plots were trampled annually since May 1995 while five plots left untrampled. Trampling was defined as two flat-footed stomps over the entire area of the plot. Soil from each plot was sampled in April 2004 (nematodes) and May 2004 (protozoa) at 10 to cm increments: 0 to 10 cm from the shallow, medium, and deep profile, 10 to 20 cm from the medium and deep profile, and 20 to 30 cm from the deep profile. Nematodes were enumerated by the cotton filter method (see Chapter 2) from duplicate subsamples of 150 to 200 g and protozoa were enumerated from 9 g with the most probable number method (Chapter 2) for all 60 samples: 2 treatments (trampled or non-trampled), 5 replicate plots, and 6 profile/depths (0 to 10 cm for shallow, 0 to 10 and 10 to 20 cm for medium, and 0 to 10, 10 to 20, and 20 to 30 cm for deep). A mean of 150 individuals per experimental unit were identified to genus.

Analysis

Only the Crust Removal Experiment was analyzed with univariate analysis, but both experiments were analyzed with multivariate analysis. The univariate dependent variables analyzed in the Crust Removal Experiment included nematode, amoebae,

flagellate, and ciliate biomass, and the ecological indices of richness, diversity, maturity, and bacterivorous nematodes as a proportion of all nematodes. Biomass for each group of microfauna was estimated from abundance by assuming dry mass conversion rates of 0.10 µg per nematode, 0.0000848 µg per amoebae, 0.0000106 µg per flagellate, and 0.000636 µg per ciliate (Petersen and Luxton, 1982; Griffiths et al., 1995). Diversity (Shannon, 1948) at the genus level was computed as $\sum p_i \ln(p_i)$, where p_i is the proportion of each genus i (n_i/N). Finally, the combined Maturity Index (Bongers, 1990, as modified by Yeates, 1994) was computed as the weighted mean of colonizer-persister (cp) values: $\sum p_i \cdot cp_i$, where p_i is the proportion of each all genera i and cp_i is the cp -value of genus i . Genera from families thought to represent faster-growing, colonizer-type individuals are assigned a low cp -value (1 or 2) and genera from families thought to represent slower-growing, persister-type individuals are assigned a high cp -value (4 or 5). Consequently, low maturity values represent a community dominated by fast-growing, colonizer-type individuals while high maturity values represent a community with relatively more slow-growing, persister-type individuals. To meet assumptions of normality, nematode, amoebae, flagellate, and ciliate biomass was \log_{10} transformed [$\log_{10}(x)$] prior to analysis, bacterivorous nematodes as a proportion of all nematodes was arcsine of square-root transformed [$\sin^{-1}(\sqrt{p})$] prior to analysis, and diversity and maturity index values were not transformed because they were Gaussian. Dependent variables were analyzed with mixed linear models using the MIXED procedure of SAS Version 9.1 (SAS Institute, Inc., Cary, NC) in two phases. First, profile type (shallow, medium, and deep),

treatment (trampled or non-trampled) and the two-way interaction was tested as completely randomized for 0 to 10 cm samples only. Secondly, depth (nested within profile type) was tested for medium and deep profile types only. Means comparisons were made by Fisher's protected Least Significant Difference (LSD) Test: if treatment effects were found to be significant ($p < 0.05$) then effect means were compared by studentized t -tests, if treatment effects were not found to be significant then no means comparisons were made.

Multivariate analysis of nematode community composition from the Plant Experiment used a modification of the Principal Response Curves (PRC) approach of Van den Brink and Ter Braak (1998, 1999). The PRC method is a multivariate ordination approach that modifies Redundancy Analysis (RDA) to accommodate repeated measures data and visualize community composition over a time-series. Typically, PRC diagrams compare composition of treatment communities versus some control community over time using least-squares species weights obtained from the RDA. The present application used space in place of the 'time-series' as the x -axis. The 'control' was defined as far interspace microsites pooled from both crust types and represented as 'aggregate far interspace community'. Crust types were compared at each microsite (stem, dripline, 3, 10, and 35 cm). The resulting diagram illustrates community response as a function of species composition across microsites in comparison to the aggregate far interspace community. Quantitatively, the formula $f_{kt} = c_{dt} \cdot b_k$ fits the modeled abundance of species k at microsite t as a fraction f_{kt} of the log-abundance of species k relative to the

control (pooled far interspace samples) where c_{dt} is the overall community response of treatment d at microsite t and b_k is the weighting factor for species k . Similarly, $f_{kt} = \exp(c_{dt} \cdot b_k)$ quantifies the fraction f_{kt} of the geometric mean of non-transformed original abundance to the same parameters as above. Data were log-transformed prior to analysis and the initial RDA was computed using CANOCO software Version 4.5 (Biometris, Wageningen, The Netherlands). The significance of the first axis of the RDA model was tested against 499 unrestricted Monte Carlo permutations.

Multivariate analysis of nematode community composition from the Crust Removal Experiment included direct gradient correspondence analysis (CCA) to identify patterns of association between nematode community composition, physical trampling, and soil profile type. All genera were regarded as species variables and the six treatment combinations (i.e., 3 soil profiles x 2 trampling treatments = 6) were coded as nominal variables (1, 0) and used as the environmental variables. Data were log-transformed prior to analysis and CCA was computed using CANOCO software. The significance of the first axis and the full model was tested against 499 unrestricted Monte Carlo permutations.

Results

Plant Experiment

Nematode community composition shifted towards the plant stem as a result of most, but not all, genera increasing in abundance nearer to the plant (Figure 10, 11). Nematode community composition in proximity to plants was most different between

crust types in I-Sky communities, where the cyano/lichen/moss crusts contained disproportionately more individuals of the genera that are characteristic of the stem microsites. The first axis of these models were significant for three of the four locations and plant type community types, including shrubs in I-sky ($p = 0.0020$), shrubs in Needles ($p = 0.0300$), and grasses in Needles ($p = 0.0160$), but not grasses in I-Sky ($p = 0.4600$).

Crust Removal Experiment

Nematodes and flagellates were more abundant at the surface (0 to 10 cm) of the deep profile than the medium or shallow profile and only nematodes, but no protozoan groups, were affected negatively by trampling (Table 4). Nematodes and protozoa were generally, but not always, more abundant at the surface (0 to 10 cm) than at depth (10 to 20 cm and 20 to 30 cm) (Table 5). Most notably, amoebae at the surface (0 to 10 cm) were less abundant (but statistically not different from) subsurface (10 to 20 cm) soil in the medium profile (Table 5). Richness of nematode genera was greater beneath non-trampled plots than trampled ($p = 0.0186$) and bacterivores were a larger portion of the nematode community beneath trampled plots than non-trampled plots ($p = 0.0021$), but Maturity Index values did not differ between trampled and non-trampled plots ($p = 0.1553$) (Table 6). Richness, diversity, ecological maturity, and bacterivores were greater at the surface than at depth for both the medium and deep profiles (Table 7).

Nematode composition of the three profiles was diametrically opposed to each other in the first two CCA axes (Figure 12). Eigenvalues of CCA axis 1 (0.176, $p =$

0.0020) and axis 2 (0.083) explained 75.6 % of the total species-environment variance.

The species-environment correlations were large for axis 1 (0.975) and axis 2 (0.919).

Discussion

Some genera increase in abundance nearer to plants disproportionately more (such as the enrichment bacterivore *Panagrolaimus*) or less (such as *Stegelletina*, *Acromoldavicus*, or *Aphelenchus*, depending on the plant type of interest), than others. I hypothesize that this is a consequence of a subtle difference in desiccation tolerance between species of the most diverse group of desert nematodes, the bacterivorous Cephalobidae. Darby et al. (2007) found different nematode communities between shallow (0 to 10 cm) and deep (10 to 30 cm) soil at both the cool desert (Colorado Plateau) and hot desert (Chihuahuan Desert) location. The majority of this distinction was due to relatively more fast-growing, desiccation tolerant colonizer-type nematodes at the surface but more slower-growing, persister-type nematodes at depth that are relatively intolerant of desiccation. However, there were also differences, though less dramatic, between species of the family Cephalobidae. *Acromoldavicus* was absent from Chihuahuan Desert, but in the Colorado Plateau *Acromoldavicus* was relatively more abundant at the surface while *Acrobeles* was relatively more abundant at depth. In the Plant Experiment, *Acromoldavicus* increased nearer to the plant disproportionately less than other genera around the grass *Stipa* in Island in the Sky Park and around both *Stipa* and the shrub *Coleogyne* in Needles Park. Conversely, *Acrobeles* increased close to the plant disproportionately more than other genera associated with either plant type at both

locations. Thus, I hypothesize that this is evidence of competition at least between the confamilial genera *Acrobeles* and *Acromoldavicus*, where *Acromoldavicus* is more tolerant to rapid desiccation and prolonged drought than *Acrobeles*. Accordingly, *Acromoldavicus* will outcompete with *Acrobeles* at the surface, prone to rapid desiccation, and in the plant interspaces, with less organic matter to slow desiccation.

The most notable finding of the Crust Removal study was that not only did crust removal (physical trampling) decrease nematode abundance, richness, and diversity, but trampling shifts the nematode microbivore trophic structure towards a greater dominance of bacterivores. This supports Belnap (1995), who found that a similar trampling disturbance resulted in a 40 to 60 % decrease in the ratio of total fungi to total bacteria, depending on the location studied. Similarly, the abundance of fungivorous nematodes was reduced with disturbance more than bacterivorous nematodes. A comparison of reported means suggests that trampling disturbance caused a greater reduction in fungivores than bacterivores. In our study, bacterivores represented 53% of the community in non-trampled plots and up to 64% of the community in trampled plots. A shift in microbivores from fungivores to bacterivores mirrors the shift in energy channels observed in agricultural food webs after some physically disruptive management practices. For example, annual tillage practices led to a dominance of bacterial energy channels over fungal channels in comparison to no-till (Hendrix et al., 1986) or integrated management (Moore and De Ruiter, 1991) practices.

I hypothesize that one reason nematodes were affected adversely by crust removal more than protozoa is that protozoa may have been able to utilize a broader range of prey. More than nematodes, protozoa are recognized as being able to access part of their energy from phagocytizing not just bacteria but also dissolved and particulate organics. Protozoa were not enumerated past broad motility groupings, but additional experimentation with a finer taxonomic resolution may also reveal that many protozoa were predaceous on nematodes. Finally, nematodes are several orders of magnitude greater in biomass than most protozoa, and I hypothesize that body size may also contribute to the tolerance of protozoa to crust removal. The physical trampling employed here may have increased bulk density, reduced porosity, and constricted the habitable pore spaces available to nematodes more than protozoa.

In conclusion, while nematodes are generally more abundant near plants than in the interspaces, the impact of desert plants on soil microfauna appears to be a combination of carbon input, microclimate amelioration, and altered soil hydrology. Physical trampling of desert soil appears to decrease the abundance of nematodes but not protozoa, and shifts the decomposition energy channel towards the bacterial pathway. This is important because the bacterial decomposition channel is thought to be faster and associated with greater nitrogen mineralization than the fungal decomposition channel (Ingham et al., 1985). Greater rates of nitrogen mineralization could lead to large losses of total nitrogen in a system prone to losses gaseous and liquid nitrogenous compounds (Peterjohn and Schelsinger 1990).

References.

- Belnap, J. (1995). Surface disturbances: Their role in accelerating desertification. *Environmental Monitoring and Assessment*, 37(1-3), 39-57.
- Belnap, J. (2002). Impacts of off road vehicles on nitrogen cycles in biological soil crusts: Resistance in different US deserts. *Journal of Arid Environments*, 52(2), 155-165.
- Belnap, J. (2002). Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biology and Fertility of Soils*, 35(2), 128-135.
- Belnap, J. (2003). The world at your feet: Desert biological soil crusts. *Frontiers in Ecology and the Environment*, 1(4), 181-189.
- Belnap, J., & Gillette, D. A. (1997). Disturbance of biological soil crusts: Impacts on potential wind erodibility of sandy desert soils in southeastern Utah. *Land Degradation & Development*, 8(4), 355-362.
- Belnap, J., & Gillette, D. A. (1998). Vulnerability of desert biological soil crusts to wind erosion: The influences of crust development, soil texture, and disturbance. *Journal of Arid Environments*, 39(2), 133-142.
- Belnap, J., Phillips, S. L., & Miller, M. E. (2004). Response of desert biological soil crusts to alterations in precipitation frequency. *Oecologia*, 141(2), 306-316.
- Belnap, J., & Warren, S. D. (2002). Patton's tracks in the Mojave Desert, USA: An ecological legacy. *Arid Land Research and Management*, 16(3), 245-258.
- Beymer, R. J., & Klopatek, J. M. (1991). Potential contribution of carbon by microphytic crusts in pinyon-juniper woodlands. *Arid Soil Research and Rehabilitation*, 5(3), 187-198.
- Bongers, T. (1990). The maturity index: An ecological measure of environmental disturbance based on nematode species composition. *Oecologia*, 83, 14-19.
- Evans, R. D., & Belnap, J. (1999). Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. *Ecology*, 80(1), 150 to 160.
- Evans, R. D., & Johansen, J. R. (1999). Microbiotic crusts and ecosystem processes. *Critical Reviews in Plant Sciences*, 18(2), 183-225.
- Ferris, H., Bongers, T., & De Goede, R. G. M. (2001). A framework for soil food web diagnostics: Extension of the nematode faunal analysis concept. *Applied Soil Ecology*, 18(1), 13-29.
- George, D. B., Roundy, B. A., St Clair, L. L., Johansen, J. R., Schaalje, G. B., & Webb, B. L. (2003). The effects of microbiotic soil crusts on soil water loss. *Arid Land Research and Management*, 17(2), 113-125.
- Griffiths, B. S., Young, I. M., & Caul, S. (1995). Nematode and protozoan population-dynamics on decomposing barley leaves incubated at different soil matrix potentials. *Pedobiologia*, 39(5), 454-461.
- Hendrix, P. F., Parmelee, R. W., Crossley, D. A., Jr., Coleman, D. C., Odum, E. P., & Groffman, P. M. (1986). Detritus food webs in conventional and no-tillage agroecosystems. *BioScience*, 36(6), 374-380.

- Housman, D. C., Yeager, C. M., Darby, B. J., Sanford, R. L., Kuske, C. R., Neher, D. A., et al. (2007). Heterogeneity of soil nutrients and subsurface biota in a dryland ecosystem. *Soil Biology & Biochemistry*, 39(8), 2138-2149.
- Hu, C. X., Liu, Y. D., Song, L. R., & Zhang, D. K. (2002). Effect of desert soil algae on the stabilization of fine sands. *Journal of Applied Phycology*, 14(4), 281-292.
- Ingham, R. E., Trofymow, J. A., Ingham, E. R., & Coleman, D. C. (1985). Interactions of bacteria, fungi, and their nematode grazers: Effects on nutrient cycling and plant growth. *Ecol. Mono.*, 55(1), 119-140.
- Jeffries, D. L., Klopatek, J. M., Link, S. O., & Bolton, H. (1992). Acetylene-reduction by cryptogamic crusts from a blackbrush community as related to resaturation and dehydration. *Soil Biology & Biochemistry*, 24(11), 1101-1105.
- Moore, J. C., & de Ruiter, P. C. (1991). Temporal and spatial heterogeneity of trophic interactions within below-ground food webs. *Agriculture, Ecosystems and Environment*, 34, 371-397.
- Peterjohn, W. T., & Schlesinger, W. H. (1990). Nitrogen loss from deserts in the southwestern United States. *Biogeochemistry*, 10(1), 67-79.
- Petersen, H., & Luxton, M. (1982). A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos*, 39, 288-388.
- Reynolds, R. L., Belnap, J., Reheis, M., Lamothe, P., & Luiszer, F. (2001). Aeolian dust in Colorado Plateau soils: Nutrient inputs and recent change in source. *Proceedings of the National Academy of Sciences*, 98, 7123-7127.
- Rosentreter, R., & Belnap, J. (2001). Biological soil crusts of North America. In J. Belnap & O. L. Lange (Eds.), *Biological soil crusts: Structure, function, and management* (pp. 31-50). New York: Springer-Verlag.
- Rychert, R. C., & Skujins, J. (1974). Nitrogen fixation by blue-green algae-lichen crusts in the Great Basin Desert. *Soil Science Society of America Proceedings*, 38, 768-771.
- Rychert, R. C., Skujins, J., Sorensen, D., & Porcella, D. (1978). Nitrogen fixation by lichens and free-living microorganisms in deserts. In N. E. West & J. Skujins (Eds.), *Nitrogen in desert ecosystems* (pp. 20 to 30). Stroudsburg: Dowden, Hutchinson and Ross, Inc.
- Schlesinger, W. H., & Pilmanis, A. M. (1998). Plant-soil interactions in deserts. *Biogeochemistry*, 42(1-2), 169-187.
- Schlesinger, W. H., Raikes, J. A., Hartley, A. E., & Cross, A. E. (1996). On the spatial pattern of soil nutrients in desert ecosystems. *Ecology*, 77(2), 364-374.
- Schlesinger, W. H., Reynolds, J. F., Cunningham, G. L., Huenneke, L. F., Jarrell, W. M., Virginia, R. A., et al. (1990). Biological feedbacks in global desertification. *Science*, 247(4946), 1043-1048.
- Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, 27, 379-423, 623-656.

- Van den Brink, P. J., & Ter Braak, C. J. F. (1998). Multivariate analysis of stress in experimental systems by principal response curves and similarity analysis. *Aquatic Ecology*, 32, 163-178.
- Van Den Brink, P. J., & Ter Braak, C. J. F. (1999). Principal response curves: Analysis of time-dependent multivariate responses of biological community to stress. *Environmental Toxicology and Chemistry*, 18(2), 138-148.
- West, N. E., & Skujins, J. (1977). The nitrogen cycle in North American cold-winter semi-desert ecosystems. *Ecologia Plantarum*, 12, 45-53.
- Williams, J. D., Dobrowolski, J. P., West, N. E., & Gillette, D. A. (1995). Microphytic crust influence on wind erosion. *Transactions of the ASAE*, 38(1), 131-137.
- Yeates, G. W. (1994). Modification and qualification of the nematode maturity index. *Pedobiologia*, 38, 97-101.

Table 4. Treatment Analysis of Microfauna from Crust Removal Experiment.

(A) F-values from analysis of profile type, disturbance treatment, and the profile*treatment interaction, on \log_{10} transformed amoebae, flagellate, ciliate, and nematode biomass ($\mu\text{g kg}^{-1}$) at just 0 to 10 cm, followed by (B) comparison of least-squares means of \log_{10} transformed biomass between the three profiles (at 0 to 10 cm) and (C) trampled and control (non-trampled) treatments (0 to 10 cm).

(A)	<u>Effect</u>	<u>df</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Profile	2, 24	0.97	4.95*	3.09	6.22**
	Treatment	1, 24	2.20	0.31	2.54	4.81*
	Profile* Treatment	2, 24	2.11	0.08	1.08	2.05
(B)	<u>Profile</u>		<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Deep		2.33	0.80 ^A	2.08	2.67 ^A
	Medium		2.23	1.09 ^B	2.38	2.41 ^B
	Shallow		2.41	1.18 ^B	1.92	2.41 ^B
	(SE)		0.091	0.089	0.131	0.060
(C)	<u>Treatment</u>		<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Trampled		2.24	0.99	2.01	2.42 ^A
	Control		2.40	1.05	2.25	2.57 ^B
	(SE)		0.074	0.073	0.107	0.049

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column)

indicate contrasting means within an effect ($p < 0.05$). Means without a letter indicate a non-significant effect and no comparisons were made.

Table 5. Depth Analysis of Microfauna from Crust Removal Experiment.

(A) F-values from nested analysis of variance of trampling experiment comparing the effect of depth on log-transformed amoebae, flagellate, ciliate, and nematode biomass ($\mu\text{g kg}^{-1}$), and (B) comparison of least-squares means of log-transformed values between multiple depths for the deep and medium profiles. 0 to 10 cm values for the shallow profile are equivalent to the 'shallow' entry of Table 1(B)

(A)	<u>Effect</u>	<u>df</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Depth(Profile)	4, 45	15.37**	15.83**	15.07**	14.92**

(B)	<u>Depth</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
(Deep profile)					
	0 to 10 cm	2.33 ^A	0.80 ^{AB}	2.08 ^A	2.67 ^A
	10 to 20 cm	1.98 ^B	0.17 ^C	0.85 ^C	2.17 ^{BC}
	20 to 30 cm	1.57 ^C	0.50 ^C	0.40 ^C	1.83 ^D
(Medium profile)					
	0 to 10 cm	2.23 ^A	1.09 ^A	2.38 ^A	2.41 ^B
	10 to 20 cm	2.32 ^A	0.54 ^B	1.47 ^B	2.05 ^{CD}
	(SE)	0.082	0.108	0.212	0.084

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column) indicate contrasting means ($p < 0.05$).

Table 6. Treatment Analysis of Indices from Crust Removal Experiment.

(A) F-values from analysis of variance of trampling experiment for four ecological indices relating to nematode communities (Shannon's diversity, richness, Bonger's Maturity Index, and bacterivores) at 0 to 10 cm, followed by (B) comparison of least-squares means between the three profiles and (C) trampled and control (non-trampled) treatments.

(A)	<u>Effect</u>	<u>df</u>	<u>Diversity</u>	<u>Richness</u>	<u>Maturity</u>	<u>Bacterivores</u>
	Profile	2, 24	10.26***	10.36***	9.40**	4.72*
	Treatment	1, 24	4.00	6.37*	2.15	11.85**
	Profile* Treatment	2, 24	0.67	0.73	1.08	0.96
(B)	<u>Profile</u>		<u>Diversity</u>	<u>Richness</u>	<u>Maturity</u>	<u>Bacterivores</u>
	Deep		2.39 ^A	22.7 ^A	2.37 ^A	0.94 ^A
	Medium		2.21 ^B	18.6 ^B	2.60 ^B	0.84 ^B
	Shallow		2.00 ^C	16.3 ^B	3.32 ^A	0.84 ^B
	(SE)		0.061	1.007	0.048	0.028
(C)	<u>Treatment</u>		<u>Diversity</u>	<u>Richness</u>	<u>Maturity</u>	<u>Bacterivores</u>
	Trampled		2.13	17.7 ^A	2.39	0.93 ^A
	Control		2.27	20.7 ^B	2.47	0.82 ^B
	(SE)		0.050	0.822	0.039	0.023

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column) indicate contrasting means within an effect ($p < 0.05$). Means without a letter indicate a non-significant effect and no comparisons were made.

Table 7. Depth Analysis of Indices from Crust Removal Experiment.

(A) F-values from analysis of variance of trampling experiment comparing the effect of depth on four ecological indices relating to nematode communities (Shannon's diversity, richness, Bongers' Maturity Index, and bacterivores) and (B) comparison of least-squares means of log-transformed values between multiple depths for the deep and medium profiles.

(A)	<u>Effect</u>	<u>df</u>	<u>Diversity</u>	<u>Richness</u>	<u>Maturity</u>	<u>Bacterivores</u>
	Depth (Profile)	4, 45	5.12**	12.15***	12.26***	5.85***
(B)	<u>Depth</u>		<u>Diversity</u>	<u>Richness</u>	<u>Maturity</u>	<u>Bacterivores</u>
(Deep profile)						
	0 to 10 cm		2.39 ^A	22.7 ^A	2.37 ^B	0.94 ^A
	10 to 20 cm		2.14 ^{BC}	16.7 ^B	2.29 ^{BC}	0.82 ^B
	20 to 30 cm		1.96 ^D	13.0 ^C	2.23 ^C	0.81 ^B
(Medium profile)						
	0 to 10 cm		2.21 ^B	18.6 ^a	2.60 ^A	0.84 ^B
	10 to 20 cm		1.92 ^{CD}	14.2 ^b	2.20 ^C	0.70 ^C
	(SE)		0.085	1.102	0.046	0.035

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Contrasting letters of the same case (within a column) indicate contrasting means ($p > 0.05$).

Figure 9. Nematode Abundance and Composition around Plants.

Nematode abundance (left axes, bars) and bacterivorous nematodes (right axes, lines) surrounding a shrub (*Coleogyne*, A, C) and a grass (*Stipa*, B, D), in the Island in the Sky (A, B) and Needles (C, D) region of Canyonlands National Park. Populations were enumerated from five microsites away from the plant (stem, dripline, and 3, 10, and 35 cm from dripline) for two crust types, cyanobacteria crusts (stripped bars and dashed lines with open markers) and cyano/lichen/moss crusts (solid bars and solid lines with solid markers). Although nematodes declined in abundance away from the plant stem, the proportion of the individuals that are bacterivorous were similar statistically.

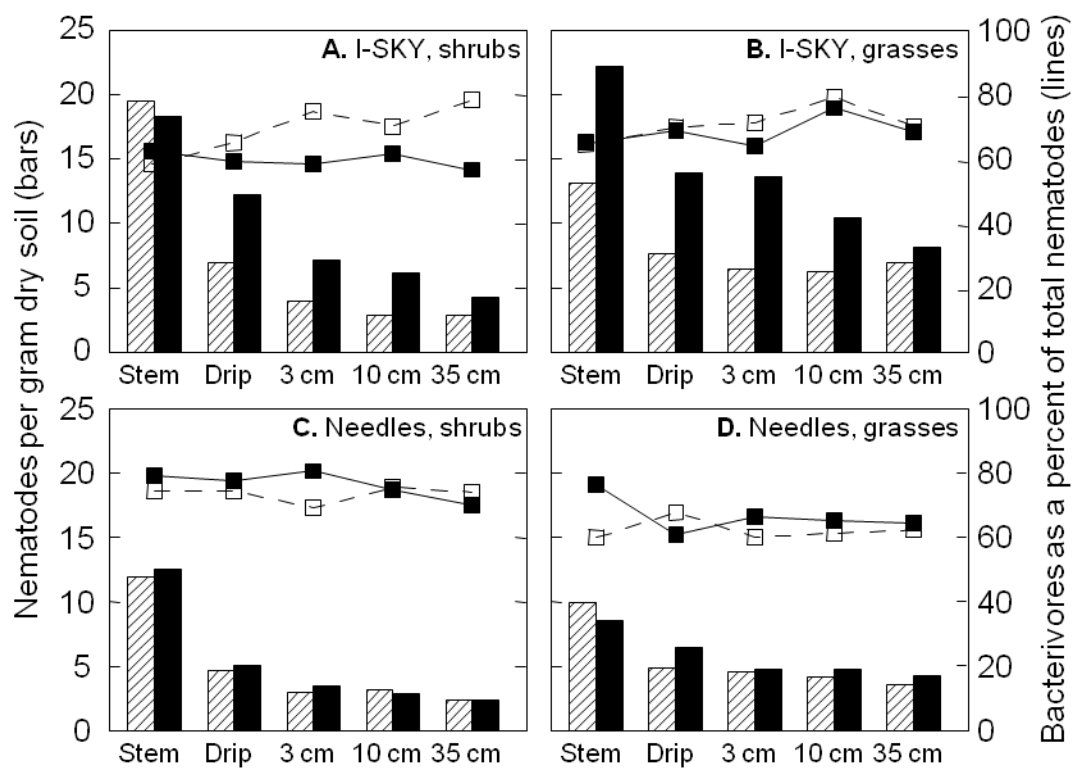


Figure 10. Island in the Sky Nematode Community Composition around Plants. Principal Response Curves of genera surrounding a grass (*Stipa*, A), and a shrub (*Coleogyne*, B) in the Island in the Sky region of Canyonlands National Park. Solid lines depict communities associated with late-successional cyano/lichen/moss crusts, while broken lines depict communities associated with early-successional cyanobacteria crusts. A shift in the overall community response (C_{dt}) to positive values represents a greater number of species with positive species weights (b_k). Species with weights between -0.5 and 0.5 were removed for clarity.

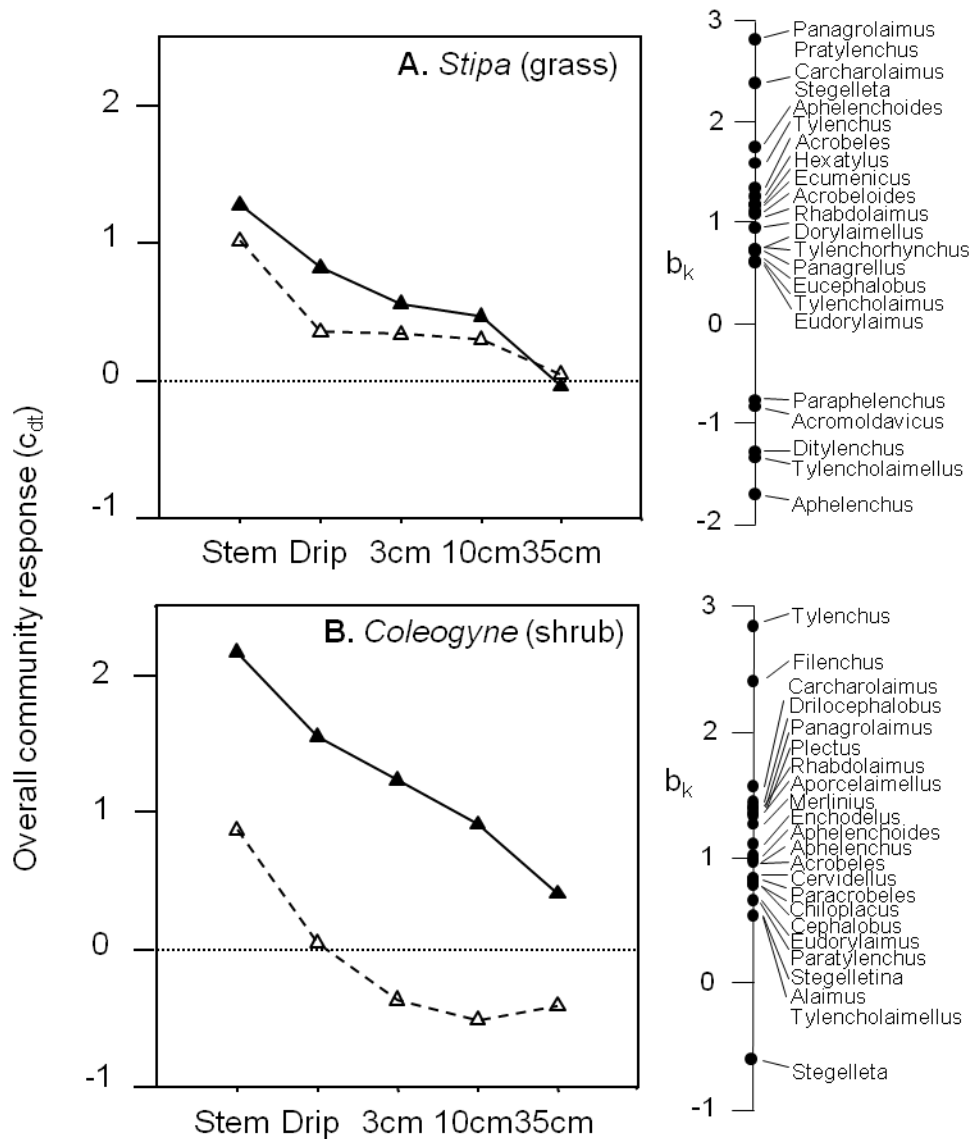


Figure 11. Needles Nematode Community Composition around Plants.

Principal Response Curves of genera surrounding a grass (*Stipa*, A), and a shrub (*Coleogyne*, B) in the Needles region of Canyonlands National Park. Solid lines depict communities associated with late-successional cyano/lichen/moss crusts, while broken lines depict communities associated with early-successional cyanobacteria crusts. A shift in the overall community response (C_{dt}) to positive values represents a greater number of species with positive species weights (b_k). Species with weights between -0.5 and 0.5 were removed for clarity.

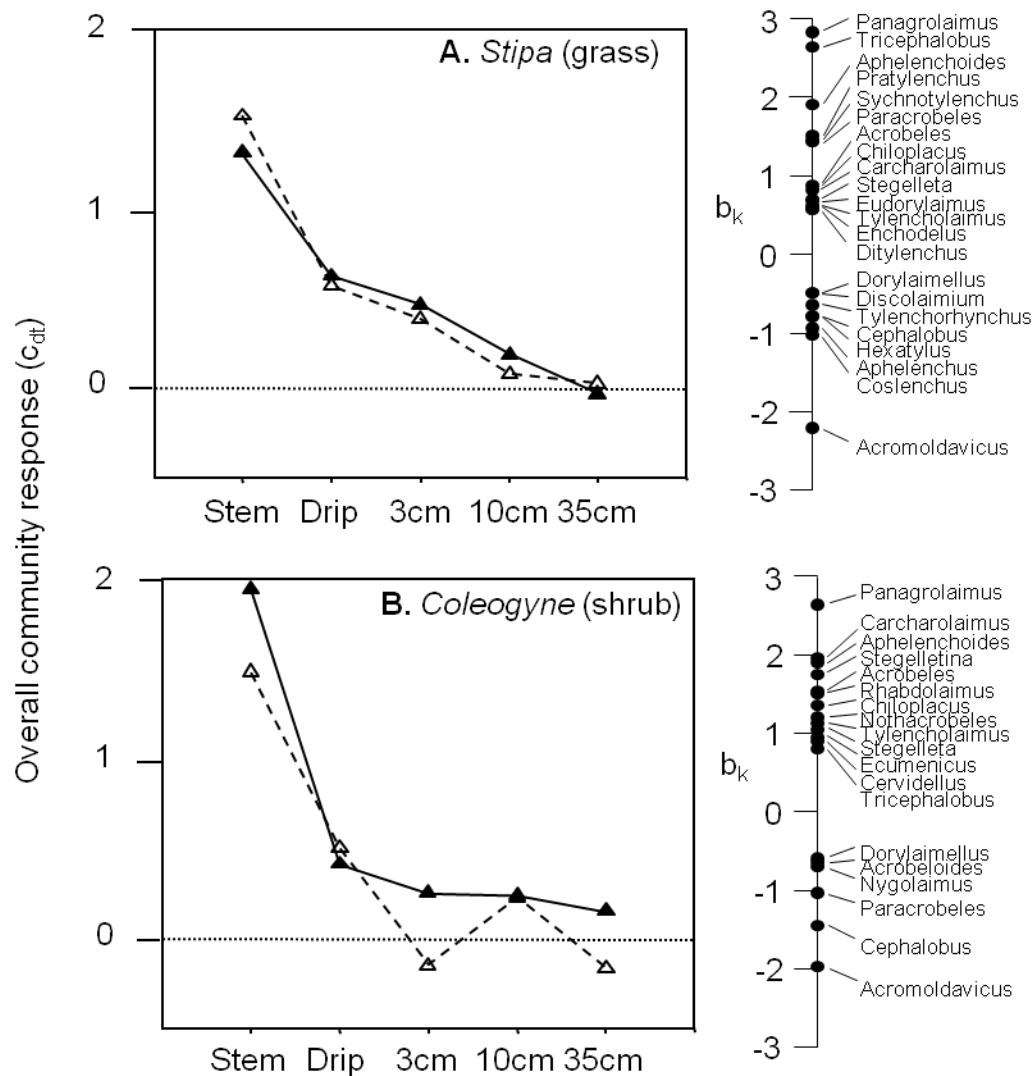
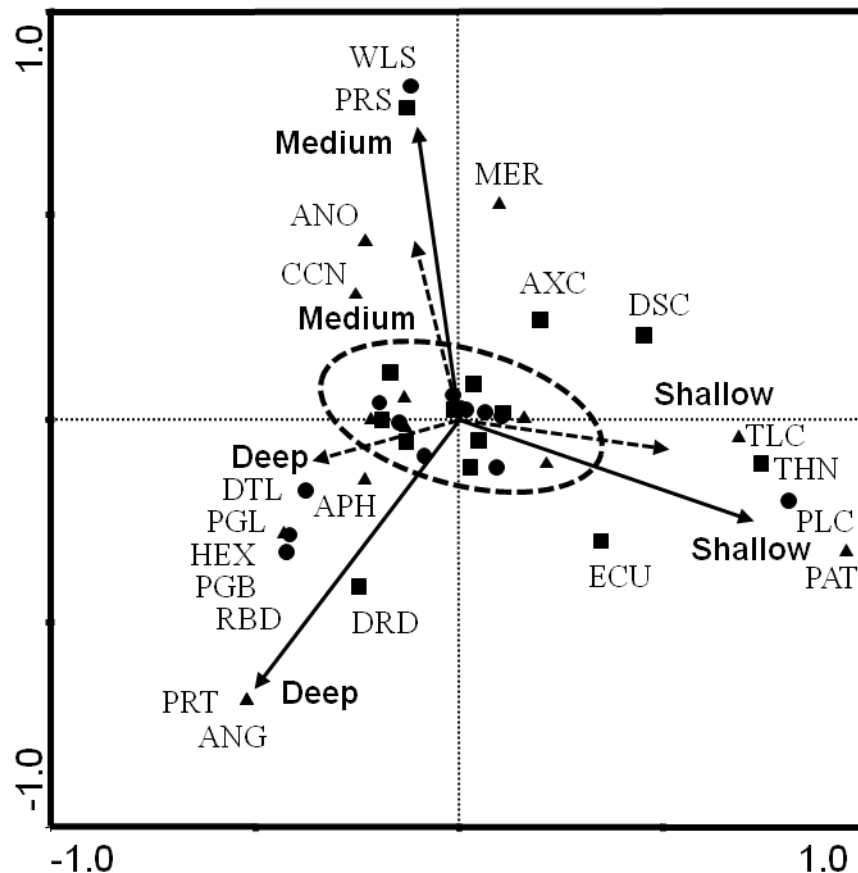


Figure 12. Nematode Community Composition from Crust Removal Experiment. Canonical Correspondence analysis of 0 to 10 cm nematodes from trampled experiment. Environmental vectors represent treatment combinations of profile depth (shallow, medium, or deep), and physical disturbance (trampled, dashed lines, or non-trampled, solid lines). Points represent relative abundance of tylenchid piercers (triangles), dorylaimid piercers (squares), and bacterivores (circles). Genera within the dashed circle were excluded for clarity, remaining genera include: ACB, Acrobeles; ANG, Anquina; ANO, Anomyctus; APC, Aporcelaimellus; APH, Aphelenchus; AXC, Axonchium; CCH, Carcharolaimus; CCN, Criconemella; CPL, Chiloplacus; DRD, Dorydorella; DSC, Discolaimium; DTL, Ditylenchus; ECU, Ecumenicus; ENC, Longidorella; FLC, Filenchus; HEX, Hexatylus; MER, Merlinius; NYG, Nygolaimus; PAC, Paracrobeles; PAT, Paratylenchus; PGB, Panagrobolium; PGL, Panagralaimus; PLC, Plectus; PRS, Pristomatolaimus; PRT, Pratylenchus; RBD, Rhabdolaimus; STG, Stegelletta; SYC, Sychnotylenchus; THN, Thonus; TLC, Tylenchus; TML, Tylencholaimellus; TRC, Tylenchorhynchus; WLS, Wilsonema;



CHAPTER 4. ENVIRONMENTAL STRESS AS A DETERMINANT OF SOIL MICROFAUNAL COMPOSITION

Desert soil communities contain at least four main groups of bacterivorous microfauna: amoebae, flagellates, ciliates and nematodes (plus occasional rotifers). These groups perform a similar trophic function, bacterivory, but contrast in size, motility, and life history traits. Cephalobidae is the most abundant and frequently occurring family of bacterivorous nematodes. Other families, including Drilocephalobidae, Rhabditidae, Panagrolaimidae, Plectidae, Alaimidae, and Monhysteridae, are typically represented only in unusually moist or nutrient-enriched microhabitats. The diversity of arid land bacterivorous nematodes is unique from many temperate regions. For example, in a tallgrass prairie, bacterivorous nematodes are represented by several families but each family contains only a few species (Jones et al., 2006). In contrast, arid soils often contain one or a few families but with many species (De Ley, 1992). Thorne (1925) reviewed over 30 species of Cephalobidae found in the arid southwest US alone, with additional species later described (Thorne, 1937; Allen and Noffsinger, 1971; De Ley et al., 1999; Baldwin et al., 2001). Thus, the desert soil habitat harbors diverse bacterivorous consumers with the potential for overlap at two conspicuous levels: the functional group level (between amoebae, flagellates, ciliates, and nematodes) and at the genus level (within the family Cephalobidae). The questions remain, “what determines

the relative composition of functional groups, and how can so many species coexist in the single family Cephalobidae?”

A variety of abiotic stressors constrain life for water-film desert soil fauna like nematodes: 1) rapid desiccation, 2) drought duration, 3) extreme temperature while anhydrobiotic and, 4) brevity of feeding time while active. Soil nematodes rely on a thin film of moisture for activity, but up-regulate synthesis of cellular protecting proteins and low-molecular weight sugars, such as trehalose and glucose, to stabilize cells and prevent membrane rupture. Upon drying, rapid desiccation of soil kills microfauna that have not been stimulated adequately in time to safely enter anhydrobiosis. Regarding the time spend anhydrobiotic, the physiological consequence of drought duration is unclear but has been documented for nematodes and rotifers (Walters, 2005). Some organisms (e.g., rotifers and protozoa) appear to survive dry dormant stages according to the ‘Sleeping Beauty’ model where organisms resume activity upon re-wetting practically un-aged (Ricci et al., 1987; Ricci and Perletti, 2006). Other organisms, such as the bacterivorous nematode *Panagrolaimus rigidus*, continue to age during dormancy and lose fecundity, according to the “Picture of Dorian Gray” model (Ricci and Pagani, 1997). Extreme temperatures, even for anhydrobiotic organisms, denature proteins despite a number of heat shock proteins and other cellular protectants formed at the initiation of anhydrobiosis. Some desert soil fauna are notorious for surviving brief periods of extreme heat, such as Rahm’s 1922 report of submitting tardigrades to 151 °C for 15 min (Crowe, 1971). However, survival at extreme temperatures drops rapidly at exposures of

hours. Finally, anhydrobiosis and subsequent rehydration is metabolically costly and can leave the organism in a negative energetic balance if the soil environment is not kept moist for a sufficiently long period of time after a rain event to graze and replace lost carbon. Although nematodes may become uncoiled and begin feeding within an hour after wetting, respiration rates are elevated throughout the re-hydration process and do not return to normal basal rates until up to two hours after re-hydration (Crowe et al., 1977).

The objective of this study is to explore what abiotic aspects of the desert soil environment determine the relative composition of functional guilds in the food web and the relative composition of species within functional guilds. My approach is to test the effect of environmental scenarios that represent major abiotic stressors of the desert soil: rapid desiccation, extreme temperature while anhydrobiotic and, and brevity of feeding time while active. I first compare the major nematode and protozoan functional guilds in general, and secondly the individual genera of a bacterivorous nematode family (i.e., Cephalobidae) in greater detail. I hypothesize that the various species and functional groups are separated from each other according to Leibold's (1995) concept of the niche. not only does niche space include the multidimensional hypervolume of space, environment, and resources that species *live in*, but also the response (i.e., growth, reproduction, and survival) of a species to a particular niche dimension and the environmental conditions that allow a species *to exist*. Thus, I hypothesize that

functionally similar species coexist as a result of unique tolerances to environmental stress.

Methods

This project features soil nematode communities from two deserts that contrast in mean annual temperature and timing of precipitation. Canyonlands National Park, in southeastern Utah (38°35.08'N, 109° 49.16'W), represents the cool desert location of the Colorado Plateau where mean annual temperature is 14.17 °C and greater than 60 % precipitation occurs in winter. Soils for this region are loamy mixed, calcareous, in the Rizno series and slightly basic (pH = 7.5 to 8.5). Jornada Experimental Range, in southern New Mexico (32°31.80'N, 106° 43.41'W), represents the hot desert location of the Chihuahuan Desert where mean annual temperature is 14.56 °C and >60 % precipitation occurs in summer. Soils for this region are classified as Regan series clay loam and are also slightly basic.

Five experiments were conducted to test the role that temperature and precipitation have on desert soil microfaunal communities. The experiments progress through the wetting and drying event and address the following five questions, the A) effect of wetting frequency on community abundance, B) interaction of elevated temperature and frequent wetting, C) effect of season on the response of nematodes to wetting, D) effect of rapid desiccation prior to anhydrobiosis, and E) effect of extreme temperature events while anhydrobiotic. The five experiments are labeled A) Wetting

Frequency, B) Wetting Temperature, C) Wetting Season, D) Rapid Desiccation, E) Extreme Temperature, respectively

A. Wetting Frequency

Ninety crust-covered soil cores were collected from the Colorado Plateau and contained in 15 cm diameter PVC rings placed in an outside rain-out shelter at ambient temperatures beginning July 2006. Ninety soil cores were assigned randomly to one of three simulated rain frequencies designed to mimic 30, 15, or 10 rain events per month, corresponding to 300%, 150%, and 100% of the 40 year average frequency, respectively. Rain events applied once every one, two, or three days were comprised of 3, 1.5, and 1 mm event sizes, respectively. Ten cores from each treatment were harvested monthly in each of three consecutive months, and an additional 10 samples were collected prior to the experiment, in June and 10 in July to estimate baseline abundance. Subsamples from cores were harvested destructively and shipped via overnight courier to University of Vermont (i.e., Burlington) and analyzed for abundance of nematodes and protozoa in 200 g and 9 g soil, respectively (Chapter 2).

B. Wetting Temperature

Fifty crust-covered soil cores were collected from the Colorado Plateau and contained in 15 cm diameter PVC rings placed in an outside rain-out shelter at ambient temperatures beginning July 2007. Ten samples were used to estimate baseline abundance of fauna, while the remaining forty cores were subjected to 40 year median timing and amount of precipitation with half the cores experiencing ambient temperature

conditions and the other half under a heating lamp to elevate temperatures 2-3 °C above ambient. Ten cores from both treatments were harvested after one month and the rest after two months. Subsamples from harvested cores were shipped via overnight courier to University of Vermont (i.e., Burlington) and analyzed for abundance of nematodes and protozoa using protozoan 200 g and 9 g soil, respectively.

C. Wetting Season

Thirty 100 to g soil samples from Colorado Plateau and thirty from Chihuahuan Desert were collected April 2004 (before summer) and again for both locations in September 2004 (before winter). Samples were shipped overnight to Toledo, Ohio (operating institution) and stored for one week at 35° C prior to the summer experiment and at 10° C prior to the winter experiment. The soil samples for the experiments were contained in PVC rings of 5 cm diameter (5 cm deep) with No-see-um screen (Rainshed, Inc., Corvallis, OR) glued to the bottom with water-fast epoxy. The PVC rings were degassed at 90° C for 48 h and sanitized in dilute bleach solution for 24 h prior to the experiments. The microcosm experimental unit consisted of 50 g soil (held at field moisture) in each ring with the rings placed on top of 2 cm of coarse gravel (approximately 1 cm diameter) at the bottom of a 150 ml beaker to allow air flow. In a fully factorial design, all samples were assigned randomly to one of two moisture regimes and one of three temperature treatments, with five replications for each treatment combination. Two moisture regimes were employed: 1) allowed to continually dry throughout the experiment (“dry”), and 2) kept at field capacity (–10 kPa) throughout the

experiment (“wet”). Soil moisture at matric potential of -10 kPa was determined by placing the microcosm rings in contact with Büchner funnels with fritted glass plates of fine porosity (Duniway, 1976). Tension funnels were saturated with degassed, deionized water and elevated to 100 cm to pull -10 kPa tension. This moisture level was maintained throughout the experiment by covering the experimental unit (beaker) with aluminum foil and by adding sterilized DI water weekly to return the soil to its wet weight at -10 kPa.

Three temperature treatments were employed to mimic seasonal average daily temperatures for three locations: 1) Colorado Plateau, 2) Chihuahuan Desert, and 3) Sonoran Desert. Each temperature treatment was applied with a diurnal thermoperiod of 16 h day: 8 h night during the summer experiments and 10 h day: 14 h night during the winter experiment. Temperatures used for the summer experiment were 1) 35°C day (25°C night), 2) 40°C day (30°C night), and 3) 50°C day (40°C night). Temperatures during the winter experiment were 1) 10°C day (0°C night), 2) 15°C day (5°C night), and 3) 25°C day (15°C night). All experimental units were incubated in sealed plastic containers and stored in their respective growth chamber for the duration of the 6-week experiment.

D. Rapid Desiccation

In January 2006, 50 crust-covered soil samples were collected from the field near Canyonlands National Park, Utah (Colorado Plateau). Each sample was divided into five 30 g subsamples and added to 5-cm diameter soil cores (1 cm thick). The subsample cores from each sample were wetted at room temperature for 10 to 12 hours and

subsequently dried in a 25 °C incubator. The subsamples were connected to a compressor and dried with a continuous flow of desiccated air and the flow rate was calibrated to reproduce 120, 30, 20, or 10 min drying times. Ten replicates of the fifty samples were allocated to each drying rate and the final ten samples served as non-wetted controls. This experiment was repeated in July 2007 to test the effect of similar drying rates but at the middle of the summer season and at a warmer temperature. Fifty crust-covered soil cores were collected from the same area and divided into subsamples as before, and wetted for two hours prior to desiccation at 35 °C at drying rates of 120, 30, and 10 min. Ten samples served as non-wetted controls and ten samples were wetted but allowed to air-dry at room temperature. After desiccation, each subsample from both experiments were shipped via overnight courier to University of Vermont (Burlington) and analyzed for nematode and protozoan abundance using 120 g (four of the five 30 to g subsamples) and 9 g (one of the five 30 to g subsamples), respectively.

E. Extreme Temperature

Five large samples of soil (>2 kg) were collected from the Canyonlands National Park field site, air dried, and sieved gently (2 mm) to remove rocks. The sample was homogenized gently in a plastic beaker by holding the beaker at a 45° angle and slowly rotating at least 10 full revolutions. The sample was split into two equal parts and each half of the sample was assigned randomly to one of two treatments: 1) control (not heated) exposed to room temperature, or 2) heated at 65°C for 48 h in an open glass beaker. Subsamples of both treatments from each sample were analyzed for nematode

and protozoan abundance using 100 g and 9 g soil, respectively, while the remaining soil was used for a separate experiment (Chapter 7).

Statistical Analysis

For all experiments, total abundance of nematodes and protozoa were \log_{10} transformed prior to analysis [$\log_{10}(x+1)$] and used as dependant variables in analysis of variance (PROC MIXED, SAS Institute, Inc., Cary, NC). Means comparisons were made by Fisher's protected Least Significant Difference (LSD) Test: if treatment effects were found to be significant then effect means were compared by studentized t -tests, if treatment effects were not found to be significant then no means comparisons were made. In the case of experiment E) Rapid Desiccation, fixed effects found statistically significant by ANOVA were analyzed with Dunnett's post-hoc tests to determine which treatment levels differed significantly from controls. Enumeration of individual nematode genera from the family Cephalobidae was performed for three of the five experiments. Wetting Frequency (A): Abundance of each genus, expressed as individuals per gram dry soil and log-transformed prior to analysis, and compared between baseline and the first month. The resulting slope was used as an estimate of monthly exponential growth rate assuming all genera are growing exponentially through the first month of the experiment. Wetting Season (C): Genera present in each experimental unit was recorded but not analyzed statistically because too few nematodes were recovered due to a relatively small sample size (30 g) and substantial mortality rates at high temperatures. Rapid Desiccation (D): The relative abundance of genera as a proportion of all individuals (a value ranging

from 0 to 1) was arcsine of square root transformed prior to analysis by ANOVA between just the baseline and 20 min desiccation treatment of both experiments. The resulting difference is an index of desiccation tolerance representing the change in relative abundance. Larger (i.e., positive) values represent genera affected less-negatively than most, while smaller (i.e., negative) values represent genera affected more negatively.

Results

A. Wetting Frequency

All wetting frequencies initially increased the abundance of all microfaunal groups (Table 8). Nematodes, flagellates, and ciliates decreased in abundance after the first month, coincident with maximum summer temperatures and a rapid decline in microbial DNA, while amoebae continued to increase through the second month (Figure 13). *Acrobeles*, *Acrobeloides*, *Acromoldavicus*, *Aphelenchus*, *Cervidellus*, *Discolaimium*, *Ecumenicus*, and *Longidorella* were among the genera with positive growth rates (Table 9).

B. Wetting Temperature

Amoebae, flagellates, and ciliates initially increased in abundance through the first month and declined in abundance the second month (Table 10). Nematodes under ambient conditions increased after both the first and the second month, but nematodes under elevated temperatures had increased only after the second month (Table 10, Figure 14).

C. Wetting Season

Elevated summer temperatures decreased total nematode abundance at both moisture levels (Figure 15). However, nematodes from both Colorado Plateau and Chihuahuan Desert were eliminated entirely from the highest temperature (50 °C) when kept wet throughout the experiment. Small populations from both locations remained in the soils allowed to dry naturally. *Acrobeloides*, *Cervidellus*, *Chiloplacus*, and *Stegelletina* were the only four genera remaining in dry microcosms incubated at the highest temperatures (50 °C) (Table 11).

D. Rapid Desiccation.

During the winter (i.e., first) portion of the desiccation rate experiment, nematodes, flagellates, and ciliates experienced 83, 64, and 70 % mortality, respectively, in all drying rates compared to non-wetted controls (Table 12). However, only the fastest drying time (10 min) caused significant mortality of amoebae. During the summer (i.e., second) portion of the desiccation rate experiment, nematodes experienced 50% mortality in the 120 min and 30 min treatments and 80% mortality in the 20 min treatment (Table 13). Flagellates and ciliates experienced 50 and 80% mortality in the 20 min treatment only. *Acromoldavicus*, *Tylenchus*, and *Aphelenchoides* were among the genera that experienced disproportionately less mortality (Table 14, Table 15).

E. Extreme Temperature.

Nematodes and flagellates experienced 45 and 30% mortality, respectively, due to the 65 °C treatment for 48 h. In contrast, amoebae and ciliates were not affected significantly (Table 16).

Discussion

Cool desert soil microfauna experience four main abiotic stresses during the summer, 1) rate at which soil dries after a rain event, 2) duration of drought events between rain events, 3) extreme temperature during drought events, and 4) duration of moisture sufficient to allow feeding during and just after a rain event. Generally, cool desert nematodes were affected more negatively than amoebae by the various stressors tested. In turn, amoebae were relatively abundant in less suitable climate conditions than nematodes. Sixteen of the 19 climate change models used by the Inter-governmental Panel on Climate Change predict elevated mean annual temperature and prolonged droughts (Seager et al., 2007). Petersen and Luxton (1982) demonstrate that nematode abundance, like that of many other soil fauna, is unimodal along a mean annual temperature gradient, i.e., most abundant in temperate regions and less abundant in extremely warm and extremely cold regions. Nematodes are relatively abundant in the Colorado Plateau as a desert, but are still much less abundant than in temperate regions. Thus, I propose three hypotheses. First, elevated temperatures will accelerate desiccation and limit grazing of bacterivorous consumers following rain events. Second, summer seasons will become more frequently stressful for microfauna than at present and that

summer mortality will offset regrowth through cool seasons. Third, nematodes will be affected adversely more than amoebae by the cumulative abiotic stresses. In short, nematodes will gradually decrease more than other protozoa (on a scale of centuries) and amoebae will likely decrease less than nematodes do. This is important because amoebae, generally three orders of magnitude less biomass per organism, are expected to respire more per biomass than nematodes. Carbon lost to respiration is thought to be linked to nitrogen lost to either cellular catabolism or mobilization of ingested prey, so a switch in microfaunal community composition could alter the nitrogen cycling function of the soil food web. For example, nitrogen mineralization could actually accelerate relative to consumed prey biomass if the flow of microbial biomass shifts from nematodes (energetically-efficient) to protozoa (energetically-inefficient).

In addition to the difference between nematodes and protozoa, the various nematodes species within a functional group were each uniquely susceptible to abiotic stresses. In comparison to the rest of the community of bacterivorous nematodes, *Acromoldavicus mojavicus* was least susceptible to rapid desiccation in the winter, *Cervidellus* and *Stegelletina* were least susceptible to prolonged extreme temperature while anhydrobiotic, and *Acrobeloides* grew most rapidly with frequent wetting. In previous studies (Darby et al., 2007), *Acrobeles* and *Acromoldavicus mojavicus* were most abundant at Colorado Plateau (i.e. cool desert) where most of the precipitation occurs during the winter. *Acrobeloides*, *Cervidellus* and *Stegelletina* were most abundant at Chihuahuan Desert (i.e., hot desert) characterized by greater mean annual temperature

where more precipitation occurs during the summer than the winter. The coarse textured soil at the Colorado Plateau site drains quickly while the fine textured soil at the Chihuahuan site drains slowly. This suggests that the nematode community from both sites originate from a similar post-glacial community but have shifted subsequently to mostly *Acrobeles* and *Acromoldavicus* at the Colorado Plateau, representative of fast drying and prolonged drought soils, and to mostly *Acrobelloides*, *Cervidellus*, and *Stegelletina* at the Chihuahuan Desert, representative of high summer temperatures and slow drying fine textured soils.

While nematodes were found to be affected more adversely by the abiotic stresses applied in these studies, it is important to note that the treatments applied here generally exaggerate what is typically experienced in the field. For example, 50°C is experienced in these soils, but only at the very surface and not for 12 h as applied in experiment C. The mortality of microfauna observed at the highest temperature treatment in experiment C is an exaggeration of what is predicted to occur in the field only after summer precipitation events on very warm summer days. Similarly, desiccation rates of 120, 30, 20, and 10 min are representative of only the surface soils, so the mortality of microfauna observed at these desiccation rates in experiment D is applicable in the field only for nematodes at 0 to 5 cm. Thus, additional laboratory experiments are needed to test more realistic rates of abiotic stress at subsurface levels (5-15 cm), but will need to employ effective means of managing the natural variability of soil biota populations to accurately distinguish among treatment means. Furthermore, field experiments are also needed to test the

response of soil microfauna to abiotic stress in field conditions where the abiotic stresses are more realistic. Depending on the ambient climate of such field experiments, abiotic stress may not necessarily induce mortality of intolerant taxa, but instead restrain population growth relative to tolerant taxa.

In conclusion, nematodes were affected more negatively by the most common abiotic stresses than amoebae, and various nematodes each showed tolerance or intolerance to a different suite of abiotic stresses. In the desert soil environment, abiotic stress functions as a local rarefying agent that maintains diversity by selectively removing species, and allowing for the growth of others, when both stress and opportunity are heterogeneous in space and time. However, a homogenization of abiotic stress could shift the community toward species tolerant to that stress and even permanently remove species from the regional species pool. Thus, functional redundancy within a trophic level does not negate the value of biodiversity. Rather, functionally redundant species are a valuable 'insurance' to the mortality of intolerant species (Loreau et al., 2003). On a fine scale, microfauna are susceptible to different abiotic stresses based on the nature of the pore space they inhabit. Individuals inhabiting large pores, hydrologically isolated pores, or pores near the soil surface, whether by chance or by preference, are more susceptible to mortality by rapid desiccation, prolonged drought, or extreme temperatures, respectively. A diverse local species pool could facilitate re-growth of total community biomass by recolonizing spaces made vacant by abiotic stress.

References

- Allen, M. W., & Noffsinger, E. M. (1971). *Nothacrobeles*, n. gen., with descriptions of four new species (Nematoda: Cephalobidae). *Journal of Nematology*, 3(2), 145-153.
- Baldwin, J. G., De Ley, I. T., Mundo-Ocampo, M., De Ley, P., Nadler, S. A., & Gebre, M. (2001). *Acromoldavicus mojaviensis* n. sp. (Nematoda : Cephalobidae) from the Mojave Desert, California. *Nematology*, 3, 343-353.
- Crowe, J. H. (1971). Anhydrobiosis: An unsolved problem. *The American Naturalist*, 105(946), 563-573.
- Crowe, J. H., Madin, K. A. C., & Loomis, S. H. (1977). Anhydrobiosis in nematodes: Metabolism during resumption of activity. *Journal of Experimental Zoology*, 201, 57-64.
- De Ley, I. T., De Ley, P., Baldwin, J. G., Mundo-Ocampo, M., & Nadler, S. A. (1999). Three new species of *Nothacrobeles* (Nemata: Cephalobidae) from the Mojave Desert, California. *Journal of Nematology*, 31(4), 482-497.
- De Ley, P. (1992). The nematode community of a marginal soil at Cambérène, Senegal, with special attention to functional morphology and niche partitioning in the family Cephalobidae. *Mededelingen van de Koninklijke Academie voor Wetenschappen, Letteren en Schone Kunsten van België - Klasse der Wetenschappen*, 53, 108-153.
- Duniway, J. M. (1976). Movement of zoospores of *Phytophthora cryptogea* in soils of various textures and matric potentials. *Phytopathology*, 66(7), 877-883.
- Jones, K. L., Todd, T. C., Wall-Beam, J. L., Coolon, J. D., Blair, J. M., & Herman, M. A. (2006). Molecular approach for assessing responses of microbial-feeding nematodes to burning and chronic nitrogen enrichment in a native grassland. *Molecular Ecology*, 15(9), 2601-2609.
- Leibold, M. A. (1995). The niche concept revisited: Mechanistic models and community context. *Ecology*, 76(5), 1371-1382.
- Loreau, M., Mouquet, N., & Gonzalez, A. (2003). Biodiversity as spatial insurance in heterogeneous landscapes. *Proceedings of the National Academy of Sciences of the United States of America*, 100(22), 12765-12770.
- Petersen, H., & Luxton, M. (1982). A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos*, 39, 288-388.
- Ricci, C., & Pagani, M. (1997). Desiccation of *Panagrolaimus rigidus* (Nematoda): Survival, reproduction and the influence on the internal clock. *Hydrobiologia*, 347, 1-13.
- Ricci, C., & Perletti, F. (2006). Starve and survive: Stress tolerance and life-history traits of a bdelloid rotifer. *Functional Ecology*, 20(2), 340 to 346.
- Ricci, C., Vaghi, L., & Manzini, M. L. (1987). Desiccation of rotifers (*Macrotrachela quadricornifera*) - survival and reproduction. *Ecology*, 68(5), 1488-1494.

- Seager, R., Ting, M. F., Held, I., Kushnir, Y., Lu, J., Vecchi, G., et al. (2007). Model projections of an imminent transition to a more arid climate in southwestern North America. *Science*, 316(5828), 1181-1184.
- Thorne, G. (1925). The genus *Acrobeles* von Linstow, 1887. *Transactions of American Microscopical Society*, 44(4), 171-210.
- Thorne, G. (1937). A revision of the nematode family Cephalobidae, Chitwood and Chitwood, 1934. *Proceedings of the Helminthological Society of Washington*, 4(1), 1-16.
- Walters, C., Hill, L. M., & Wheeler, L. J. (2005). Dying while dry: Kinetics and mechanisms of deterioration in desiccated organisms. *Integrative and Comparative Biology*, 45(5), 751-758.

Table 8. Analysis of Microfauna from Wetting Frequency Experiment.

(A) F-values from completely randomized ANOVA of amoebae, flagellate, ciliate, and nematode abundance [individuals g^{-1} , $\log_{10}(x+1)$] from the Wetting Frequency experiment. (B) Monthly means of \log_{10} transformed amoebae, flagellate, ciliate, and nematode abundance.

(A)	<u>Effect</u>	<u>df</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Month	2, 100	2.46	4.28*	0.67	5.27**
	Frequency	2, 100	0.99	1.20	1.73	0.51
	Month*Freq.	4, 100	0.07	0.90	0.80	1.01

(B)	<u>Month</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	0	3.50	3.06 ^{AB}	2.48	0.60 ^C
	1	3.92	3.20 ^A	2.74	0.86 ^A
	2	4.02	3.19 ^A	2.66	0.77 ^{AB}
	3	3.97	3.04 ^B	2.69	0.69 ^{BC}
	(SE)†	0.032	0.042	0.051	0.035

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column)

indicate contrasting means within an effect ($p < 0.05$). Means without a letter indicate a non-significant effect and no comparisons were made.

†Standard Error for amoebae, flagellates, ciliates, and nematodes at time 0 = 0.039, 0.052, 0.062, and 0.043, respectively, due to a different number of baseline than treatment samples.

Table 9. Growth Rates of Nematode Genera.

Monthly exponential growth rate estimated as the difference in \log_e -transformed means $[\ln(x+1)]$ from baseline to the first month of the Wetting Frequency experiment, ranked in order of t -value ($df = 49$). Negative growth rates may not necessarily represent a decline in absolute abundance, but rather an artifact of reduced frequency (and therefore artificial ‘zeros’) due to increased minimum detection limits from an increased overall abundance.

<u>Genus</u>	<u>Growth rate</u>	<u>t-value</u>	<u>$p < t$</u>
<i>Acrobeloides</i>	6.27	9.35	<0.0001
<i>Acrobeles</i>	6.97	9.24	<0.0001
<i>Acromoldavicus</i>	7.63	9.02	<0.0001
<i>Cervidellus</i>	5.27	8.58	<0.0001
<i>Chiloplacus</i>	3.38	6.27	<0.0001
<i>Stegelletina</i>	1.98	3.26	0.0020
<i>Heterocephalobus</i>	-0.66	-1.31	0.1973
<i>Plectus</i>	-0.96	-1.87	0.0677
<i>Drilocephalobus</i>	-1.08	-2.16	0.0356
<i>Stegelleta</i>	-1.45	-3.40	0.0013
<i>Placodira</i>	-1.91	-5.59	<0.0001
<i>Panagrolaimus</i>	-2.00	-6.06	<0.0001
<i>Paracrobeles</i>	-2.00	-6.62	<0.0001
<i>Nothacrobeles</i>	-2.11	-6.87	<0.0001

Table 10. Analysis of Microfauna from Wetting Temperature Experiment.

(A) F-values from completely randomized ANOVA of amoebae, flagellate, ciliate, and nematode abundance [$\log_{10}(x+1)$] from Wetting Temperature experiment, followed by (B) comparison of least-squares means of \log_{10} to transformed amoebae, flagellate, ciliate, and nematode abundance between the three levels of the month effect.

A)	<u>Effect</u>	<u>df</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Month	1, 45	98.37***	7.26**	16.94***	4.59*
	Heat	1, 45	0.51	1.33	0.76	4.92*
	Month*Heat	1, 45	0.10	0.51	0.00	0.29
B)	<u>Month</u>		<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	0		3.76 ^A	3.06 ^A	2.17 ^A	0.61 ^A
	1		4.18 ^B	3.42 ^B	2.84 ^B	0.65 ^A
	2		3.30 ^B	3.18 ^A	2.28 ^A	0.79 ^B
	(SE)†		0.063	0.065	0.095	0.050

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column) indicate contrasting means within an effect ($p < 0.05$).

†Standard Error for amoebae, flagellates, ciliates, and nematodes at time 0 = 0.090, 0.091, 0.135, and 0.070, respectively, due to a different number of baseline than treatment samples.

Table 11. Presence of Nematode Genera after Elevated Temperatures.
Frequency of recovery (out of a maximum five replicates) of genera from dry microcosms throughout the summer growth chamber experiment.

<u>Genus</u>	<u>Utah</u>			<u>New Mexico</u>		
	35 °C	40 °C	50 °C	35 °C	40 °C	50 °C
<i>Acrobeles</i>	4	5		1	1	
<i>Acrobeloides</i>			1	3	2	
<i>Acromoldavicus</i>	4	1				
<i>Cephalobus</i>	1			1		
<i>Cervidellus</i>	4	4	3	1		1
<i>Chiloplacus</i>	4	5	3		2	
<i>Eucephalobellus</i>					1	
<i>Drilocephalobus</i>	2	2				
<i>Stegelletina</i>	4	5	2			1
<i>Plectus</i>				1		

Table 12. Analysis of Microfauna from Winter Rapid Desiccation Experiment.

(A) F-values from completely randomized ANOVA of amoebae, flagellate, ciliate, and nematode abundance [$\log_{10}(x+1)$] from first Rapid Desiccation experiment (performed through winter at 25°C). (B) Mean values of \log_{10} transformed abundance following each desiccation rate treatment.

(A)	<u>Effect</u>	<u>df</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Treatment	4, 45	3.32*	6.72***	4.22**	12.28***

(B)	<u>Treatment</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Baseline†	3.41	2.99	1.78	0.77
	120	3.05	2.55*	0.60*	-0.10***
	30	3.18	2.45**	1.08	-0.27***
	20	3.19	2.57*	0.20**	0.18**
	10	2.93**	2.31***	0.53*	-0.08***
	(SE)	0.098	0.098	0.300	0.250

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

† Represents the control by with remaining treatments were compared by Dunnett's means comparison.

Table 13. Analysis of Microfauna from Summer Rapid Desiccation Experiment.

(A) F-values from completely randomized ANOVA of amoebae, flagellate, ciliate, and nematode abundance [$\log_{10}(x+1)$] from second Rapid Desiccation experiment (performed through summer at 35 °C). (B) Mean values of \log_{10} transformed abundance following each desiccation rate treatment.

(A)	<u>Effect</u>	<u>df</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Treatment	4, 44	4.52**	2.50	3.93**	13.48***

(B)	<u>Treatment</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Baseline†	3.72	3.06	2.30	0.52
	Natural	4.02*	3.10	2.44	0.50
	120	3.67	3.11	2.30	0.17**
	30	3.87	3.17	2.22	0.19*
	20	3.17	2.83	1.51*	-0.16***
	(SE)	0.080	0.082	0.181	0.095

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

† Represents the control by with remaining treatments were compared by Dunnett's means comparison.

Table 14. Effect of Rapid Desiccation in Winter on Nematode Genera.

Effect of desiccation rate through winter, 25 °C Rapid Desiccation experiment. Effect = change in relative abundance; positive values represent relatively unaffected genera, negative values represent genera affected negatively. Ranked in order of *t*-value.

<u>Genus</u>	<u>Effect</u>	<u><i>t</i>-value</u>	<u>$p < t$</u>
<i>Acromoldavicus</i>	0.1537	1.18	0.2535
<i>Rhabditid</i>	0.007754	0.89	0.3872
<i>Acrobeles</i>	0.02033	0.2	0.8467
<i>Panagralaimus</i>	-0.04532	-0.66	0.518
<i>Placodira</i>	-0.00638	-1.13	0.2764
<i>Chiloplacus</i>	-0.05479	-1.46	0.1636
<i>Panagrellus</i>	-0.01456	-1.7	0.1077
<i>Plectus</i>	-0.04003	-2.04	0.0584
<i>Stegelletina</i>	-0.07092	-2.26	0.0378
<i>Acrobeloides</i>	-0.1229	-3.53	0.0028
<i>Cervidellus</i>	-0.2202	-3.57	0.0026
<i>Drilocephalobus</i>	-0.1094	-3.77	0.0017

Table 15. Effect of Rapid Desiccation in Summer on Nematode Genera.

Effect of desiccation rate through summer, 35 °C Rapid Desiccation experiment. Effect = change in relative abundance; positive values represent relatively unaffected genera, negative values represent genera affected negatively. Ranked in order of *t*-value.

<u>Genus</u>	<u>Effect</u>	<u><i>t</i>-value</u>	<u>$p < t$</u>
<i>Chiloplacus</i>	0.03387	1.5	0.1521
<i>Plectus</i>	0.01167	1	0.3306
<i>Stegelletina</i>	0.04698	0.83	0.4179
<i>Cervidellus</i>	0.03059	0.47	0.6447
<i>Acromoldavicus</i>	-0.08066	-0.86	0.3998
<i>Acrobeloides</i>	-0.06405	-1.24	0.2324
<i>Acrobeles</i>	-0.09122	-1.29	0.2132

Table 16. Analysis of Microfauna from Extreme Temperature Experiment.

(A) F-values from completely randomized ANOVA of amoebae, flagellate, ciliate, and nematode biomass [$\log_{10}(x)$] from Extreme Temperature experiment. (B) Mean values of \log_{10} transformed abundance following each temperature treatment.

(A)	<u>Effect</u>	<u>df</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Treatment	1, 14	0.17	4.14	0.31	48.05***

(B)	<u>Treatment</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Control	2.67	1.17	2.30	2.93 ^A
	Heated	2.73	1.02	2.26	2.65 ^B
	SE	0.073	0.064	0.062	0.122

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column)

indicate contrasting means within an effect ($p < 0.05$). Means without a letter indicate a non-significant effect ($p > 0.05$) and no comparisons were made.

Figure 13. Growth of Microfauna During Frequent Wetting.

Growth of amoebae, flagellates, ciliates, and nematodes (left axis, note logarithmic scale) through three months of periodic wetting in the Wetting Frequency experiment. Ambient temperature throughout the experiment (right axis) was obtained from MesoWest (<http://www.met.utah.edu/mesowest/>). Microbial DNA (ug g⁻¹, left axis) was obtained from Shannon Johnson and Cheryl R. Kuske (unpublished data) from identical experimental units.

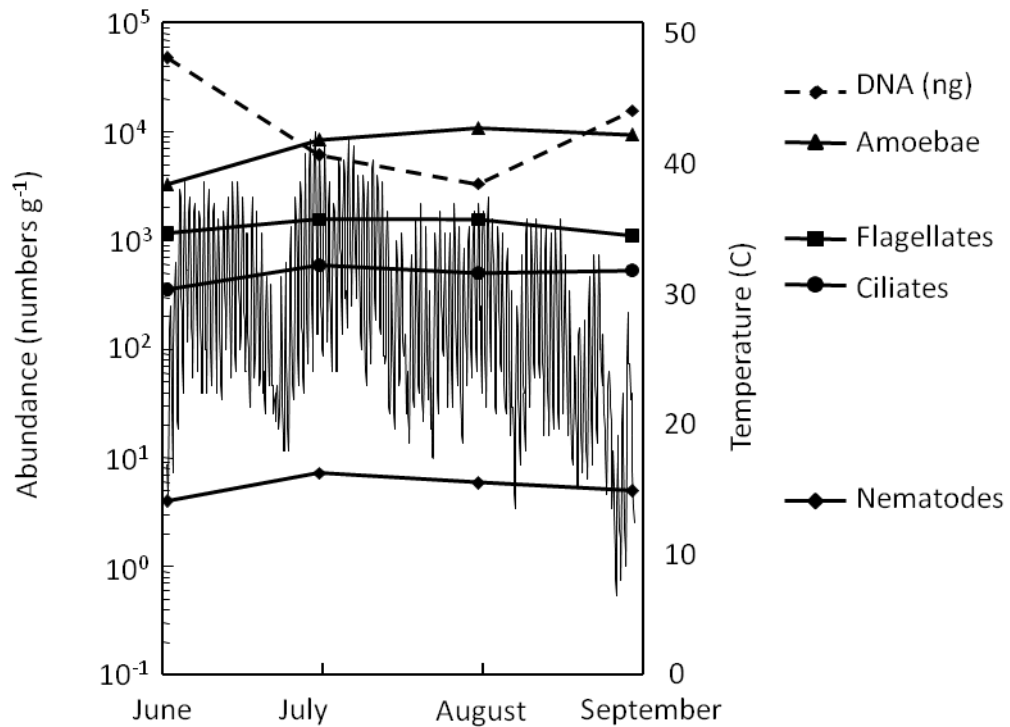


Figure 14. Growth of Nematodes with Elevated Temperature.

Growth of nematodes, expressed as the mean of \log_{10} to transformed values, through two months of periodic wetting from the Wetting Temperature experiment. Control treatments were left at ambient conditions, while heated treatments were exposed to a heating lamp that raised temperature 2-3°C above ambient.

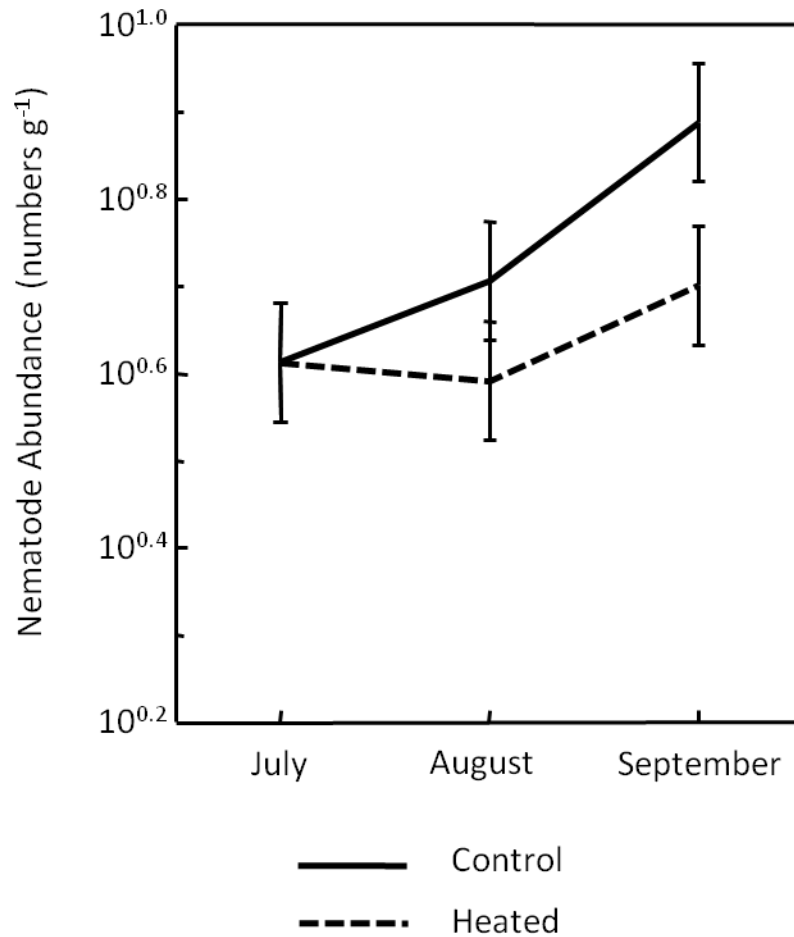
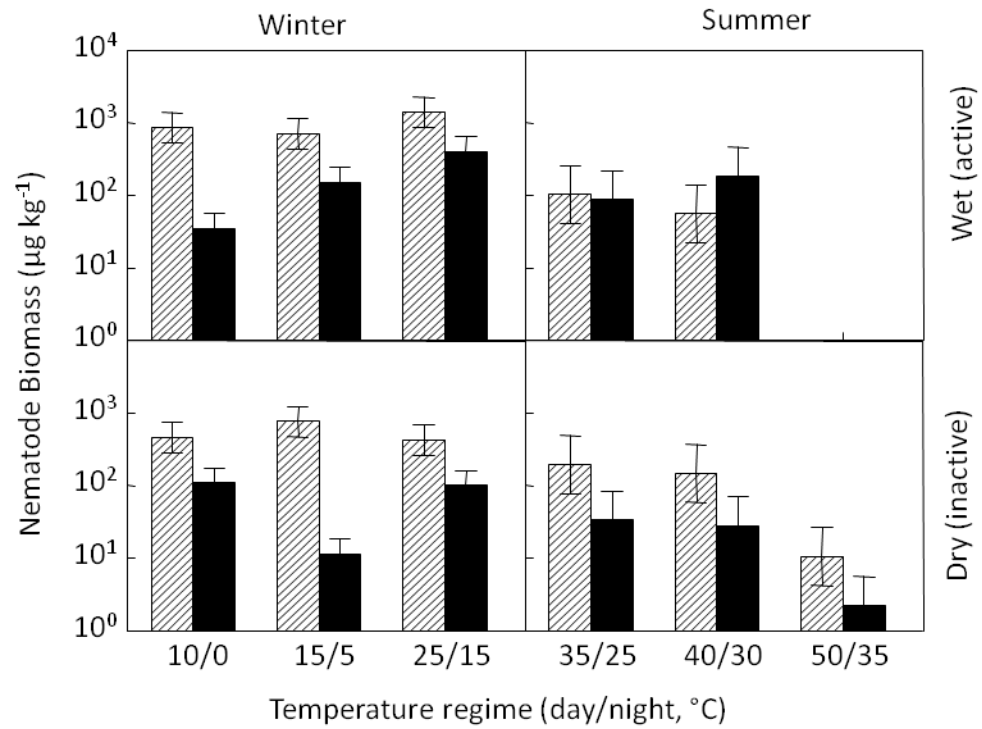


Figure 15. Nematode Biomass after Incubation at Various Temperatures.

Nematode biomass of soil from Utah (shaded bars) and New Mexico (solid bars) collected in the spring (right two graphs) and fall (left two graphs) after six weeks incubation at various temperature treatments either wet (-10 kPa, top two graphs) or dry (lower two graphs). Error bars represent one standard error, note \log_{10} to scale.



CHAPTER 5. FIELD TEST OF THE EFFECT OF INCREASED SUMMER TEMPERATURE AND PRECIPITATION ON SOIL MICROFAUNA.

Desertification is the collective process of reduced productivity in arid and semi-arid lands. Long-term grazing, exotic shrub invasion, and climate warming have been discussed as mechanisms of desertification (Schlesinger et al., 1990). However, biological soil crusts may slow desertification in many desert soil ecosystems by supplying carbon and nitrogen inputs and mediating soil hydrology, surface stability, and mineral chelation. Biological soil crusts in hot deserts of the southwest US are comprised mostly of lichens and cyanobacteria, while cool desert crusts contain a greater biomass of mosses. The composition of biological soil crusts is influenced by temperature and precipitation conditions (Belnap et al., 2006) as well as soil texture, mineralogy, and micronutrients (Bowker et al., 2005). The photosynthetic and nitrogen fixing function of crust microflora is constrained to adequate temperature and moisture conditions (Belnap, 2002; Belnap et al., 2004) and thought to be affected adversely by the predicted climate changes for many arid lands (Belnap 2003). Elevated temperature is a robust prediction for most arid lands, and 16 of the 19 models used by the Inter-governmental Panel on Climate Change predict overall drying of the southwestern US (Seager et al., 2007).

Diverse microfauna such as nematodes, protozoa, tardigrades, and rotifers mediate nutrient cycling in arid soils by consuming microflora and excreting organic and

inorganic wastes. These microfauna require a small amount of moisture to remain active. Depending on the organism, moisture requirements range from entirely water-filled pores to merely thin water films or high relative humidity. Most groups of soil microfauna feed on saprotrophs like fungi and bacteria and contribute to nutrient cycling by regulating prey biomass and, in turn, decomposition (Santos and Whitford, 1981; Santos et al., 1981; Parker et al., 1984). Soil microfauna also regulate the nitrogen cycle by the nitrogenous waste they excrete. The portion of consumed prey that is not assimilated into biomass is defecated in various stages of decomposition ranging from monomers to low molecular weight polymers to undigested particulate organics, depending on the nutrient status of the consumer (Anderson et al., 1983). The portion of consumed prey that is assimilated becomes biomass. Ammonium is the end product of protein catabolism but is toxic to most cells in high concentrations and must be exported. Terrestrial animals transport nitrogenous wastes across membranes as urea and uric acid. Soil microfauna, like many aquatic invertebrates, export most of their nitrogenous wastes as water-soluble ammonium (Wright, 1995). However, it appears that soil nematodes do produce enzymes for a functional urea cycle and export some of their nitrogenous wastes as amino acids (Wright, 1975a, b). Furthermore, rates of nitrogen cycling appear to be greatest when grazers, such as nematodes and protozoa, consume relatively high-nitrogen content prey, such as bacteria and cyanobacteria (Ingham et al., 1985; Hunt et al., 1987; Osler and Sommerkorn, 2007). The feeding anatomy of fungivorous and herbivorous soil fauna also allows feeding on cyanobacteria. For example, amoebae have filopodia, nematodes

piercing stylets, and mites piercing/rasping chelicerae. Consequently, many desert soil fauna believed to feed on fungi and plant roots in a temperate habitat may also feed on cyanobacteria in a desert soil.

Desert nematodes and protozoa enter dormant stages (i.e., anhydrobiosis or encystment, respectively) when soil moisture becomes limiting (Freckman, 1983), signaled by a combination of environmental cues such as osmolarity (Wharton, 2003; Yamaoka et al., 2004) and intracellular pH (Westh and Ramlov, 1991). Nematodes and protozoa respond quickly to wetting events by rehydrating or excysting, respectively. Abundance of nematodes and protozoa will increase rapidly under moderate temperatures and prolonged soil moisture (Chapter 4, present work; Steinberger et al., 1984; Steinberger and Sarig, 1993; Alon and Steinberger, 1999; Whitford et al., 1986; Parker et al., 1984, Freckman et al., 1987). However, it is possible that summer precipitation events could cause mortality if temperatures were sufficiently warm and soils dried rapidly after a rain event to cause both thermal and desiccation stress. This non-additive interaction of temperature and precipitation has been documented for the surface microflora (Belnap et al., 2004) but not for nematodes and protozoa in field conditions. However, Freckman et al. (1986) observed a reduction in nematode abundance coincident with late-summer monsoons in the Chihuahuan Desert. Previous controlled experiments suggest that temperature and desiccation rates severe enough to cause stress and/or mortality of soil nematodes and protozoa do occur in the field (Chapter 4), but only occur in extreme circumstances.

The primary objective of this study is to test if realistic elevated temperature and summer precipitation affects soil nematodes and protozoa negatively in field conditions as it does surface microflora. A secondary objective of this study is to estimate the contributions of each major functional group to nitrogen cycling with a soil food web model approach.

Methods

Experimental Design

A study site (60 m by 60 m) was selected near Moab, Utah (38.67485 N, -109.4163 W, 1310 m), representative of the Colorado Plateau. The soil at this site is a sandy loam mixed, calcareous, in the Rizno series (Table 17). Vascular plant vegetation at this site, comprising 5 to 20 % of the total cover, is dominated by the grasses *Hilaria*, *Stipa*, and *Bromus*. Biological soil crusts at this site, comprising up to 70 to 90 % of the total cover, is dominated by the lichen *Collema tenax*, the cyanobacterium *Microcoleus vaginatus*, and the moss *Syntrichia* sp. A randomized block design was employed where five replicate blocks were arranged perpendicular to slope. Each block contained five experimental units that were each a 2-m by 2-m plot containing representative cover. One of five treatments was applied randomly to each plot within a block: 1) control, 2) lamp control, 3) elevated temperature, 4) elevated precipitation frequency, and 5) both elevated temperature and precipitation frequency. All plots except for the “control” treatment were equipped with an infrared heating lamp in the center of the plot. The plots without elevated temperature treatments included the lamp shell only

while the plots with elevated temperature treatments included both the lamp shell and a working lamp (Model MRM-1208, 120V, 800 W, 6.7 A, 35 in., Kalglo Electronics Co. Inc, Bethlehem, PA). The lamps add no photosynthetically active radiation and have been used successfully to warm soils in previous climate change experiments (Harte et al., 1995; Bridgham et al., 1999; Zavaleta et al., 2003). Heating lamps have also been found to warm asymmetrically over diurnal periods (Bridgham et al., 1999), which is consistent with existing climate change models that predict narrowing diurnal temperature ranges caused by greater nighttime than daytime warming (Easterling et al., 1997, Stenchikov and Robock 1995, Stone and Weaver 2002). Thermocouples were constructed from 24 ga Type –T thermocouple wire (Omega Engineering, Inc., Stamford, CT) to record half-hourly temperature data at the canopy, soil surface, 5 cm, and 15 cm. Campbell CS616 water content reflectometer probes (Campbell Scientific, Inc., Logan, UT) were installed to measure volumetric water content at 5 and 15 cm depths every 30 min. To quantify the degree of warming caused by the heating lamps, the mean difference between elevated temperature and ambient temperature plots was computed every 30 min through the year 2006. Data were smoothed by a non-parametric local regression LOESS procedure of SAS Software (SAS Institute, Inc., Cary, NC). A smoothing parameter of 0.25 was used, indicating that 25% of the data were used to compute the slope at each time step (akin to collecting 4-day means). The frequency of summer precipitation was increased to approximately twice the 40 to year summer median with 2-mm artificial rainfall events (6 L of water per plot, or approximately the 40 to year median event size) five times per

two-week interval through summer. Simulated rainfall was provided with a watering nozzle calibrated to supply raindrop sizes appropriate for this region.

Sampling of all 25 plots for nematodes and protozoa occurred in March 2006, September 2006, May 2007, and September 2007 to represent spring and fall communities through two years. Surface (0 to 10 cm) soil was collected with approximately 8-10 cores (2.5 cm, 0 to 10 cm depth) to obtain at least 250 g soil for the nematode and protozoa assay. On each sampling date, cores were collected from within an 8-cm wide segment of the downslope half of the plot and each successive sampling was performed on segments adjacent to but not overlapping previous sampling segments. This was done so that all soil throughout the duration of the experiment was collected from beneath undisturbed crust surface. Sampling was restricted to rain-free days to ensure that most individuals were dormant at the time of sampling. Samples were shipped to Burlington, Vermont by overnight courier. Upon receipt, nematodes and protozoa were extracted, counted, and enumerated to genus as described previously (Chapter 2). Abundance of fauna was converted to biomass by assuming dry biomass conversion rates of 0.10 μg per nematode, 0.0000848 μg per amoebae, 0.0000106 μg per flagellate, and 0.000636 μg per ciliate (Petersen and Luxton, 1982; Griffiths et al., 1995). Additionally, amoebae were identified to genus from samples collected throughout the experiment but not analyzed statistically (Page, 1988; Rogerson and Patterson, 2000).

An index of cumulative growth potential was computed to compare seasonal population dynamics of microfauna based on the effects of temperature and moisture.

The proportion of organisms expected to be active (A) was computed as a logistic function (Figure 1): $\text{Logit}(A) = \log(A / 1-A) = \alpha + \beta\theta$ where θ represents volumetric water content, α and β are coefficients of a line.

Parameter values for the intercept ($\alpha = -2.6$) and slope ($\beta = 55.0$) were obtained by fitting the data of Freckman and Mankau (1986) by logistic regression (Figure 16). This formula estimates that 50% of the community should be active at $-\alpha/\beta = 4.7\%$ volumetric water content, which is consistent with independently collected data (Chapter 2). The relative growth rate (G , day^{-1}) of the active community was estimated using the sigmoidal expansion of the analytical function for asymmetric growth of a range of temperatures (T , $^{\circ}\text{C}$) given by Logan et al. (1976):

$$G(T) = \alpha \left(\frac{1}{1 + ke^{-\rho T}} - \exp\left(-\frac{T_M - T}{\Delta T}\right) \right)$$

where ρ is analogous to a Q_{10} value for biochemical reactions, T_M is the temperature maximum of growth, ΔT is the maximum temperature of growth (in number of degrees above T_M), and α and k are empirical constants. Parameters for the growth rate function were obtained by fitting the data for amoebae and flagellate growth (Darby et al., 2006) with non-linear regression: $\alpha = 1.19$, $k = 8.287$, $\rho = 0.150$, $T_M = 32.12$ and $\Delta T = 6.3$ (Figure 16). A growth potential index (P) was computed for each half hour interval as the product of the proportion active and relative growth rate: $P = A \times G$. A cumulative growth potential index was computed by summing over the entire range of half-hour measurements for each sampling interval. This index is a proxy for the equivalent number of days with maximum growth of a hypothetical generic soil microinvertebrate. Positive

values environmental conditions associated with growing populations and negative values suggest unfavorable environmental conditions and, thus, predict declining populations.

Nematode communities were characterized with two indices. First, bacterivorous nematodes were computed as a proportion of all individuals without a stylet or mural tooth. The remaining individuals (1 - bacterivores) represent a diverse group of individuals with piercing stylets that can prey on fungi, cyanobacteria, moss, plant roots, and/or other nematodes. Secondly, diversity was computed as $\Sigma[p_i \ln(p_i)]$, where p_i is the proportion of each genus (n_i/N) (Shannon 1948).

Statistical Analysis

Nematodes and protozoa were analyzed with mixed linear models. Six dependent variables were tested, biomass of amoebae, flagellates, ciliates, and nematodes ($\mu\text{g kg}^{-1}$) was \log_{10} transformed [$\log_{10}(x)$] prior to analysis. Bacterivores as a proportion was arcsine of square-root transformed $\{\sin^{-1}[\sqrt{p}]\}$ and genus diversity was not transformed prior to analysis. Dependent variables were tested against three independent categorical variables (and all two- and three- way interactions): temperature (ambient or elevated), precipitation (ambient or elevated), and sampling time (spring 2006, fall 2006, spring 2007, fall 2007). The experimental design was analyzed from a split-plot model (Littell et al., 1996). Field plots were blocked and temperature and precipitation applied as the whole plot factor. No difference was found between control plots with or without a ‘dummy’ lamp shell, so all plots receiving ambient temperature and ambient precipitation

were used equally as controls. Thus, the blocking design was complete but unbalanced: two controls per block and one each of all other treatment combinations. Because soil was collected from an undisturbed segment of the plot for each sampling event, sampling was not treated as repeated measures but rather as a split-plot factor. Analyses were performed using the restricted maximum likelihood method of the MIXED procedure of SAS software (Statistical Analysis Systems Institute, Inc., Cary, NC).

Nematode community composition was analyzed further with principal response curves (PRC), a multivariate ordination approach that modifies Redundancy Analysis (RDA) to accommodate repeated measures data and visualize community composition over a time-series (Van den Brink and ter Braak 1998, 1999). The visualization of PRC is a diagram that plots the multivariate community response of treated communities through time relative to control communities. Quantitatively, the formula $f_{kt} = c_{dt} \cdot b_k$ fits the modeled abundance of species k at time t as a fraction f_{kt} of the log-abundance of species k relative to the control where c_{dt} is the overall community response of treatment d at microsite t and b_k is the weighting factor for species k . Similarly, $f_{kt} = \exp(c_{dt} \cdot b_k)$ quantifies the fraction f_{kt} of the geometric mean of non-transformed original abundance to the same parameters as above. Data were log-transformed prior to analysis and the initial RDA was computed using CANOCO software Version 4.5 (Biometris, Wageningen, The Netherlands). The significance of the first axis of the RDA model was tested against 499 unrestricted Monte Carlo permutations. The reported p -value reflects the degree of fit that the RDA achieves with actual data relative to permuted (shuffled) data. For example,

when $p = 0.05$, the RDA model fit for actual data better than 95% of the models fit with permuted data.

Food Web Modeling

Cycling of carbon and nitrogen by soil fauna was estimated for this site based on the approach of Hunt et al. (1987) and de Ruiter et al. (1993). The desert food web was constructed based on best estimates of feeding habits known for the organisms found at this site (Wood, 1973, Yeates et al., 1993). Abundance of microarthropods at this site was obtained from S.A. Lewins and D.A. Neher (unpublished data). Abundance of fauna was converted to biomass by assuming dry biomass conversion rates of 1.0 μg per zoophagous or microphytophagous prostigmatid mite, 1.0 μg per oribatid mite, 2.7 μg per collembolan, 0.165 μg per tardigrade, 0.10 μg per nematode, 0.0000848 μg per amoebae, 0.0000106 μg per flagellate, and 0.000636 μg per ciliate (Petersen and Luxton 1982, Griffiths et al., 1995), 5% of biomass as nitrogen, and on a biomass per area basis to 10 cm assuming a bulk density of 1.0 g cm^{-3} (Belnap 1995). Feeding preferences and physiological parameters (Table 18) were kept as in Hunt et al. (1987) with two exceptions. First, the generation time of omnivore-predator nematodes was increased from 25 d, representative of the fast-growing *Mesodiplogaster* in prairie soils (Anderson and Coleman, 1982), to 50 d, representative of the slower-growing dorylaim omnivore-predators in the desert such as *Aporcelaimellus* (Wood, 1973). Secondly, the generation time of all protozoa was decreased from 6.67 d to 2 d (computed as the inverse of 0.5, a conservative estimate of the maximum growth rate of protozoa, Figure 1) to reflect the

fast-growing nature of protozoa found in desert soils (Bamforth 2004, 2008). As in Hunt et al. (1987), non-predatory death (D_{nat}) was estimated as the standing biomass-N (B) times turnover rate (estimating as the generation of generation time, GT , corrected for the number of days growing per year, DG):

$$D_{nat} = B \times \frac{1}{GT / DG}$$

Microarthropods were assumed to be active and growing for 365 days per year because they are thought to be active even in dry, air-filled pores. Nematodes and protozoa were assumed to be active and growing for 40 days per year. The value 40 was computed as the cumulative number of days active during 2006 from the proportion active function (A) described above, and is also the value used by Hunt et al. (1987).

Feeding rate (F_{ij}) of consumer functional group i on prey j was estimated as the sum of predatory (D_{pred}) and non-predatory death divided by the product of the fraction of prey N assimilated (e_{ass}) and the fraction of assimilated N retained as biomass (e_{prod}):

$$F = \frac{D_{nat} + D_{pred}}{e_{ass} e_{prod}}$$

Feeding rates were weighted for prey preferences as in Hunt et al. (1987), and consumed nitrogen not assimilated was allocated to substrate from feces.

Results

The site experienced slightly above mean temperature during June and July of both years, but received characteristically little precipitation (Figure 17). Precipitation at the site exceeded the 30 to year mean during March and October of 2006, but mean daily

temperatures were relatively mild during this time (i.e., 10 to 15 °C). Plots treated with heating lamps achieved 2 to 3 °C warming during evening and morning hours through much of the year (Figure 18), but heated plots were not warmer at 5 cm depth during the warmest parts of the afternoon through most of the summer (Figure 19).

Abundance of all protozoan motility groups increased through this two-year field experiment, in contrast to nematodes whose abundance remained relatively constant (Table 19, Figure 20). Effects of elevated temperature and increased precipitation altered abundance of ciliates and amoebae through time but not flagellates or nematodes. Amoebae were affected by a three-way interaction of temperature, precipitation, and sampling time. Abundance was greatest at the final summer sampling for control, elevated temperature, and elevated precipitation plots, but not for the plots receiving both elevated temperature and increased precipitation (Figure 21). Abundance of ciliates were affected by two-way interactions of temperature and sampling time, and precipitation and sampling time, but not a three-way interaction among temperature, precipitation and sampling time. Ciliates increased more during both summers in soils exposed to ambient than elevated temperatures. Ciliates in soils receiving ambient precipitation increased in abundance steadily through all sampling intervals. However, compared to ambient precipitation, ciliates responded to increased precipitation as increased abundance in the first summer and declining thereafter.

At least 47 genera and 24 families of nematodes were identified at this site over two years (Table 20), but not all genera were present every sampling season and

sampling. Nematode diversity varied by sampling time (Table 21) and was greatest when nematode abundance was also greatest (Figure 22). Conversely, the proportion of nematodes that were bacterivorous was greatest when nematode abundance was least (Table 21, Figure 20). A marginally significant three-way interaction of heat, water, and sampling for Shannon's diversity is associated with an interaction of elevated temperature and increased precipitation treatments. Plots that were both heated and watered experiencing the greatest reduction in diversity values throughout the summer sampling intervals (Figure 22). No single genus or set of genera was affected consistently by experimental treatments through time as determined by Principal response curves ($p = 0.8060$ for bacterivores, $p = 0.4560$ for stylet-bearing nematodes). At least 22 genera of amoebae were identified from this site, including *Amoeba*, *Acanthamoeba*, *Biomyxa*, *Cashia*, *Cochliopodium*, *Discameoba*, *Echinamoeba*, *Filamoeba*, *Flabellula*, *Flamella*, *Gleaseria*, *Hartmanella*, *Korotnovella*, *Mayorella*, *Platyamoeba*, *Rosculus*, *Saccamoeba*, *Stachyamoeba*, *Thecamoebae*, *Vahlkampfia*, *Vanella*, and *Vexilifera*.

Belowground soil faunal contributions to nitrogen cycling as modeled were predicted to be $311 \text{ mg N m}^{-2} \text{ yr}^{-1}$ inorganic nitrogen and $97 \text{ mg N m}^{-2} \text{ yr}^{-1}$ organic nitrogen from feces (Table 22), or $3.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ inorganic N and $1.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ organic N from feces. Most of the inorganic nitrogen was predicted to come from protozoa and bacterivorous nematodes grazing on bacteria (Table 23, Table 24). A majority of the contribution to nitrogen cycling from nematodes was predicted to be in the form of organic substrates from feces, while the majority of the contribution to

nitrogen cycling from amoebae was predicted to be inorganic from mineralization (Table 22).

Discussion

This field experiment supports two important overall conclusions, 1) desert environmental conditions restrict the growth of soil nematode populations more than protozoa, and 2) the combination of elevated summer temperature and precipitation frequency adversely affects soil fauna populations under certain conditions. Thus, the population dynamics of soil fauna must be interpreted in the context of inter-annual variability. Specifically, if the climate continues to warm as predicted (Seager et al., 2007), we can reasonably predict that summers will more frequently affect nematodes more adversely than amoebae, while winters will more frequently affect amoebae more beneficially than nematodes. Over decades and centuries, this could lead to an overall decline in standing nematode biomass but less decline, if at all, in amoebae biomass. In the absence of historical (>1000 yr) nematode abundance since the last North American glacial maxima, the scenario of declining nematode abundance is supported by observations of nematode abundance along extant temperature or topoclimatic gradients. Standing nematode biomass was found to be most abundant at moderate mean annual temperatures and less abundant at desert locations (Petersen and Luxton, 1982), and nematodes decline along a topoclimate gradient from the relatively cool, moist, high-altitude location to the relatively warmer, drier, low-altitude location (Steinberger et al., 2001). Although elevated summer precipitation frequency was the scenario tested in this

experiment, prolonged summer drought is the more common climate change prediction for the arid southwest US (Seager et al., 2007). However, amoebae tend to be more tolerant than nematodes of prolonged drought, elevated temperature which anhydrobiotic, and elevated temperature with forced to be active. I hypothesize that the prediction of decreased nematode abundance relative to amoebae is valid for both elevated summer precipitation frequency and prolonged summer drought. However, the resulting community composition would differ between the two scenarios. As discussed previously (Chapter 4), desiccation-tolerant nematodes common to cool deserts, such as *Acrobeles* and *Acromoldavicus*, would be the likely dominant genera of Cephalobidae under prolonged drought conditions. Thermo-tolerant nematodes common to hot deserts, such as *Cervidellus* and *Stegelletina*, may be the dominant genera of Cephalobidae under elevated summer precipitation frequency. Future research would benefit from 1) confirming these generalizations of stress tolerance for species throughout the arid southwest US, and 2) addressing the physiological differences between such species to help determine the functional relevance of species composition.

Two mechanisms could explain the mortality caused by summer precipitation events during high temperatures: physiological stress and desiccation rate. The temporary dormant state desert nematodes and protozoa enter upon desiccation also imparts some thermo-tolerance. However, elevated temperatures denature proteins and induces a multifaceted heat shock response that up-regulates, for example, molecular chaperones that facilitate correct folding, unfolding, and degradation of proteins (Sorensen et al.,

2003) and trehalose to reduce the damage of free-radical accumulation (Benaroudj et al., 2001). Thus, elevated temperatures during moist conditions, when nematodes are hydrated, could cause direct mortality by physiological stresses or the excess metabolism induced by the natural cellular stress responses. Alternatively, elevated temperature could accelerate soil drying after a summer rain event. Rapid desiccation of live organisms ruptures fluid membranes and exposes damaged cells. Previous experiments have shown that rapid desiccation of moist soil within as much as 120 min (a condition representative of only the very surface soil) can cause about 80% mortality of nematodes (Chapter 4). However, when moist soil is allowed to air-dry over 24 to 48 h (a condition more representative of subsurface soil) nematodes experienced no mortality and reproduced successfully. From the natural drying rates of soil in the field, desiccation would cause mortality by rapid desiccation for nematodes, but not amoebae, at the very surface (<1 cm). These conditions would most likely cause mortality by physiological stress when nematodes are moist (induced to be active) at most depths (>1-2 cm). Further research is needed to determine the relative contributions of physiological stress and metabolic carbon loss (due to responding to physiological stress) to mortality. The cumulative growth potential index did reflect the trends in nematode and flagellate abundance through each sampling interval (Table 25), but overpredicted mortality for nematodes and flagellates and was inaccurate for amoebae and ciliates. With more complete data on protozoan activity and nematode growth rates for these communities, it is likely that the

cumulative growth potential index could be calibrated to serve as a reasonable model for population dynamics of certain functional groups.

Nematodes and amoebae play different roles in soil nitrogen cycling even though both groups are dominated by bacterivorous species. Bacterivorous nematodes are assumed to have lower assimilation efficiency (0.60) than amoebae (0.95), despite similar production efficiencies (Hunt et al., 1987). According to the model, much of what bacterivorous nematodes ingest is defecated as feces. The chemistry of defecated material is important for understanding the fate of nitrogen cycling. Dissolved organic nitrogen, in the form of nitrogenous monomers such as amino acids, is a common waste product of bacterivorous nematodes (Wright et al., 1945a,b; Anderson et al., 1983) and are available for plant uptake (Schimel and Bennet, 2004). Conversely, much of what amoebae consume is assimilated as biomass and, through normal metabolic cell functions such as regulated protein turnover, is mineralized into ammonium. Microbial ammonia oxidizers are prevalent in arid soils (Johnson et al., 2005) and have the potential to rapidly oxidize ammonium into nitrate, which is prone to loss by leaching or denitrification (Cookson et al., 2006).

Two life history traits could lead to the different roles that nematodes and amoebae play in nitrogen cycling: their anatomy and size. First, bacterivorous nematodes pass their prey through a crushing valve prior to an intestinal tract that exposes crushed cellular material and non-crushed microbial cells alike to a moist, static intestinal environment that may, in time, become viewed as a humification process. Second,

amoebae are small organisms (0.08 ng ind^{-1}) that respire more carbon per unit of biomass than nematodes (100 ng ind^{-1}) (West et al., 1997, 1999). Additional physiological studies are necessary to determine whether elevated carbon demand expressed on a biomass basis will a) not affect nitrogen demand, leading to a consumed surplus and, thus, greater mobilization of non-assimilated DON, or b) accelerate metabolic nitrogen turnover and contribute to greater mineralization of assimilated nitrogen.

The consumer portion of the soil food web at this cool desert site is similar to that of the Central Plains Experimental Range (CPER) shortgrass prairie site (Hunt et al., 1987) in three general ways. First, many of the fauna respond to ephemeral pulses of activity. Second, nitrogen cycling from fauna appears to be dominated by bacterivorous nematodes and protozoa that feed on nitrogen-rich prey. Third, the role of microarthropods and higher consumer trophic levels of nematodes and protozoa may not necessarily be nitrogen cycling but rather comminution and moderating prey populations. Subtle differences in model results may be accounted for as artifacts of food web architecture, aggregation, enumeration methodology, and the choice of biological parameters. Nonetheless, there are two major ecological differences between the two food webs, 1) the presence of high-nitrogen content cyanobacteria that is preyed upon by some microbivores that would otherwise feed on low-nitrogen content fungi, and 2) a relatively greater dominance of amoebae than nematodes in the bacterivory channel. Personal observations of desert nematodes isolated from the field site support reported observations (Wood, 1973; Yeates et al., 1993) that the nematodes most likely to feed on

cyanobacteria have piercing stylets that are also capable of feeding on fungi, moss, and plant roots. For example, Wood (1973) directly observed several dorylaim nematodes (typically longer-lived, slower growing omnivores) to feed and reproduce on ‘algae’ (mixed culture of *Microcoleus* sp., *Chlorella* sp., and *Haematococcus* sp.), including the genera *Eudorylaimus* and *Aporcelaimellus*, both common in desert communities. Species of *Aphelenchus*, *Aphelenchoides*, and *Tylenchus*, also common in desert communities, were observed feeding and reproducing on moss (*Tortula princeps* and *Bryum* sp.) (Wood, 1973). The tardigrade *Haplomacrobiotus utahensis*, isolated from the field site, was observed to feed and reproduce on *Microcoleus vaginatus* and moss rhizomes (pers. obs.). Species of the nematodes *Aphelenchoides*, *Tylenchus*, and *Discolaimium* were observed feeding on the cyanobacterium *Microcoleus vaginatus*, moss rhizomes, and the cyanobacterium *Scytonema* sp., respectively (pers. obs.), but reproduction was only observed for *Aphelenchoides* feeding on *Microcoleus vaginatus* and *Tylenchus* feeding on moss.

In conclusion, I found support that 1) desert environmental conditions restrict the growth of soil nematode populations more than for protozoa, and 2) the combination of elevated summer temperature and precipitation frequency adversely affects soil fauna populations under certain conditions while elevated temperature or precipitation alone does not. Compared to temperate food webs, desert food webs are predicted to cycle a smaller quantity of nitrogen with a greater proportion of excreted nitrogen is expected to be inorganic N from amoebae than dissolved organic N from bacterivorous nematodes.

Contributions of $3.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ inorganic N and $0.98 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ organic N to substrate cycling from the fauna represent a modest proportion of the estimated $9 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ fixed biologically for a relatively undisturbed cyanobacteria crust (Belnap, 2002). Johnson et al. (2005) found that ammonium oxidation was about equal to fixation rates and that the ammonia oxidizing microbial community is oxygen, not ammonium, limited. Future research should compare the nature of nitrogen cycling between two dominant bacterivorous grazers, nematodes and amoebae. Key objectives would be to compare the chemical nature of organic nitrogen excreted through feces from both consumers, and to determine the environmental fate of the final excretory products.

References

- Alon, A., & Steinberger, Y. (1999). Response of the soil microbial biomass and nematode population to a wetting event in nitrogen-amended Negev Desert plots. *Biology and Fertility of Soils*, 30(1-2), 147-152.
- Anderson, R. V., & Coleman, D. C. (1982). Nematode temperature responses - a niche dimension in populations of bacterial-feeding nematodes. *Journal of Nematology*, 14(1), 69-76.
- Anderson, R. V., Gould, W. D., Woods, L. E., Cambardella, C., Ingham, R. E., & Coleman, D. C. (1983). Organic and inorganic nitrogenous losses by microbivorous nematodes in soil. *Oikos*, 40, 75-80.
- Bamforth, S. S. (2004). Water film fauna of microbiotic crusts of a warm desert. *Journal of Arid Environments*, 56(3), 413-423.
- Bamforth, S. S. (2008). Protozoa of biological soil crusts of a cool desert in Utah. *Journal of Arid Environments*, 72(5), 722-729.
- Belnap, J. (1995). Surface disturbances: Their role in accelerating desertification. *Environmental Monitoring and Assessment*, 37(1-3), 39-57.
- Belnap, J. (2002). Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biology and Fertility of Soils*, 35(2), 128-135.
- Belnap, J. (2003). The world at your feet: Desert biological soil crusts. *Frontiers in Ecology and the Environment*, 1(4), 181-189.
- Belnap, J., Phillips, S. L., & Miller, M. E. (2004). Response of desert biological soil crusts to alterations in precipitation frequency. *Oecologia*, 141(2), 306-316.

- Belnap, J., Phillips, S. L., & Troxler, T. (2006). Soil lichen and moss cover and species richness can be highly dynamic: The effects of invasion by the annual exotic grass *Bromus tectorum*, precipitation, and temperature on biological soil crusts in SE Utah. *Applied Soil Ecology*, 32(1), 63-76.
- Benaroudj, N., Lee, D. H., & Goldberg, A. L. (2001). Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals. *Journal of Biological Chemistry*, 276(26), 24261-24267.
- Bowker, M. A., Belnap, J., Davidson, D. W., & Phillips, S. L. (2005). Evidence for micronutrient limitation of biological soil crusts: Importance to arid-lands restoration. *Ecological Applications*, 15(6), 1941-1951.
- Bridgham, S. D., Pastor, J., Updegraff, K., Malterer, T. J., Johnson, K., Harth, C., et al. (1999). Ecosystem control over temperature and energy flux in northern peatlands. *Ecological Applications*, 9(4), 1345-1358.
- Cookson, W. R., Muller, C., O'Brien, P. A., Murphy, D. V., & Grierson, P. F. (2006). Nitrogen dynamics in an Australian semiarid grassland soil. *Ecology*, 87(8), 2047-2057.
- Darby, B. J., Housman, D. C., Zaki, A. M., Shamout, Y., Adl, S. M., Belnap, J., et al. (2006). Effects of altered temperature and precipitation on desert protozoa associated with biological soil crusts. *Journal of Eukaryotic Microbiology*, 53(6), 507-514.
- de Ruiter, P. C., van Veen, J. A., Moore, J. C., Brussaard, L., & Hunt, H. W. (1993). Calculation of nitrogen mineralization in soil food webs. *Plant and Soil*, 157, 263-273.
- Easterling, D. R., Horton, B., Jones, P. D., Peterson, T. C., Karl, T. R., Parker, D. E., et al. (1997). Maximum and minimum temperature trends for the globe. *Science*, 277(5324), 364-367.
- Freckman, D. W. (1983). Survival mechanisms in nematodes. *Journal of Nematology*, 15(4), 479.
- Freckman, D. W., & Mankau, R. (1986). Abundance, distribution, biomass and energetics of soil nematodes in a northern Mojave Desert ecosystem. *Pedobiologia*, 29(2), 129-142.
- Freckman, D. W., Whitford, W. G., & Steinberger, Y. (1987). Effect of irrigation on nematode population-dynamics and activity in desert soils. *Biology and Fertility of Soils*, 3(1-2), 3-10.
- Harte, J., Torn, M. S., Chang, F. R., Feifarek, B., Kinzig, A. P., Shaw, R., et al. (1995). Global warming and soil microclimate - results from a meadow-warming experiment. *Ecological Applications*, 5(1), 132-150.
- Hunt, H. W., Coleman, D. C., Ingham, E. R., Ingham, R. E., Elliott, E. T., Moore, J. C., et al. (1987). The detrital food web in a shortgrass prairie. *Biology and Fertility of Soils*, 3, 57-68.

- Ingham, R. E., Trofymow, J. A., Ingham, E. R., & Coleman, D. C. (1985). Interactions of bacteria, fungi, and their nematode grazers: Effects on nutrient cycling and plant growth. *Ecol. Mono.*, 55(1), 119-140.
- Johnson, S. L., Budinoff, C. R., Belnap, J., & Garcia-Pichel, F. (2005). Relevance of ammonium oxidation within biological soil crust communities. *Environmental Microbiology*, 7(1), 1-12.
- Littell, R. C., Milliken, G. A., Stroup, W. W., & Wolfinger, R. D. (1996). *SAS system for mixed models*. Cary, NC: SAS Institute, Inc.
- Logan, J. A., Wollkind, D. J., Hoyt, S. C., & Tanigoshi, L. K. (1976). An analytic model for description of temperature dependent rate phenomena in arthropods. *Environmental Entomology*, 5(6), 1133-1140.
- Osler, G. H. R., & Sommerkorn, M. (2007). Toward a complete soil C and N cycle: Incorporating the soil fauna. *Ecology*, 88(7), 1611-1621.
- Page, F. C. (1988). *A new key to freshwater and soil gymnamoebae*. Ambleside, Cumbria, UK: Freshwater Biological Association.
- Parker, L. W., Freckman, D. W., Steinberger, Y., Driggers, L., & Whitford, W. G. (1984). Effects of simulated rainfall and litter quantities on desert soil biota - soil respiration, microflora, and protozoa. *Pedobiologia*, 27(3), 185-195.
- Parker, L. W., Santos, P. F., Phillips, J., & Whitford, W. G. (1984). Carbon and nitrogen dynamics during the decomposition of litter and roots of a Chihuahuan Desert annual, *Lepidium lasiocarpum*. *Ecological Monographs*, 54(3), 339-360.
- Petersen, H., & Luxton, M. (1982). A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos*, 39, 288-388.
- Rogerson, A., & Patterson, D. J. (2000). The naked ramicristate amoebae (Gymnamoebae). In J. J. Lee, G. F. Leedale & P. Bradbury (Eds.), *The illustrated guide to the protozoa, 2nd ed.* (pp. 1023-1052). Lawrence: Allen Press.
- Santos, P. F., Phillips, J., & Whitford, W. G. (1981). The role of mites and nematodes in early stages of buried litter decomposition in a desert. *Ecology*, 62(3), 664-669.
- Santos, P. F., & Whitford, W. G. (1981). The effects of microarthropods on litter decomposition in a Chihuahuan Desert ecosystem. *Ecology*, 62(3), 654-663.
- Schimel, J. P., & Bennett, J. (2004). Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*, 85(3), 591-602.
- Schlesinger, W. H., Reynolds, J. F., Cunningham, G. L., Huenneke, L. F., Jarrell, W. M., Virginia, R. A., et al. (1990). Biological feedbacks in global desertification. *Science*, 247(4946), 1043-1048.
- Seager, R., Ting, M. F., Held, I., Kushnir, Y., Lu, J., Vecchi, G., et al. (2007). Model projections of an imminent transition to a more arid climate in southwestern North America. *Science*, 316(5828), 1181-1184.
- Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, 27, 379-423, 623-656.
- Sorensen, J. G., Kristensen, T. N., & Loeschcke, V. (2003). The evolutionary and ecological role of heat shock proteins. *Ecology Letters*, 6(11), 1025-1037.

- Steinberger, Y., Freckman, D. W., Parker, L. W., & Whitford, W. G. (1984). Effects of simulated rainfall and litter quantities on desert soil biota - nematodes and microarthropods. *Pedobiologia*, 26(4), 267-274.
- Steinberger, Y., Liang, W. J., Savkina, E., Meshi, T., & Barness, G. (2001). Nematode community composition and diversity associated with a topoclimatic transect in a rain shadow desert. *European Journal of Soil Biology*, 37(4), 315-320.
- Steinberger, Y., & Sarig, S. (1993). Response to soil nematode populations and the soil microbial biomass to a rain episode in the hot, dry Negev Desert. *Biol. Fertil. Soils*, 16, 188-192.
- Stenchikov, G. L., & Robock, A. (1995). Diurnal asymmetry of climatic response to increased CO₂ and aerosols: Forcings and feedbacks. *Journal of Geophysical Research-Atmospheres*, 100(D12), 26211-26227.
- Stone, D. A., & Weaver, A. J. (2002). Daily maximum and minimum temperature trends in a climate model. *Geophysical Research Letters*, 29(9).
- Van den Brink, P. J., & Ter Braak, C. J. F. (1998). Multivariate analysis of stress in experimental systems by principal response curves and similarity analysis. *Aquatic Ecology*, 32, 163-178.
- Van den Brink, P. J., & Ter Braak, C. J. F. (1999). Principal Response Curves: Analysis of time-dependent multivariate responses of biological community to stress. *Environmental Toxicology and Chemistry*, 18(2), 138-148.
- West, G. B., Brown, J. H., & Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science*, 276(5309), 122-126.
- West, G. B., Brown, J. H., & Enquist, B. J. (1999). The fourth dimension of life: Fractal geometry and allometric scaling of organisms. *Science*, 284(5420), 1677-1679.
- Westh, P., & Ramlov, H. (1991). Trehalose accumulation in the tardigrade *Adorybiotus coronifer* during anhydrobiosis. *Journal of Experimental Zoology*, 258(3), 303-311.
- Wharton, D. A. (2003). The environmental physiology of antarctic terrestrial nematodes: A review. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, 173(8), 621-628.
- Whitford, W. G., Steinberger, Y., Mackay, W., Parker, L. W., Freckman, D., Wallwork, J. A., et al. (1986). Rainfall and decomposition in the Chihuahuan Desert. *Oecologia*, 68(4), 512-515.
- Wood, F. H. (1973). Biology of *Aporcelaimellus* sp. (Nematoda: Aporcelaimidae). *Nematologica*, 19, 528-537.
- Wood, F. H. (1973). Feeding relationships of soil-dwelling nematodes. *Soil Biol. Biochem.*, 5, 593-601.
- Wright, D. J. (1975). Elimination of nitrogenous compounds by *Panagrellus redivivus*, Goodey, 1945 (Nematoda: Cephalobidae). *Comparative Biochemistry and Physiology. B, Comparative Biochemistry*, 52B, 247-253.

- Wright, D. J. (1975). Studies on nitrogen catabolism in *Panagrellus redivivus*, Goodey, 1945 (Nematoda: Cephalobidae). *Comparative Biochemistry and Physiology. B, Comparative Biochemistry*, 52B, 255-260.
- Wright, P. A. (1995). Nitrogen-excretion - 3 end-products, many physiological roles. *Journal of Experimental Biology*, 198(2), 273-281.
- Yamaoka, M., Watoh, T., & Matsuoka, T. (2004). Effects of salt concentration and bacteria on encystment induction in ciliated protozoan *Colpoda* sp. *Acta Protozoologica*, 43(2), 93-98.
- Yeates, G. W., Bongers, T., de Goede, R. G. M., Freckman, D. W., & Georgieva, S. S. (1993). Feeding habits in soil nematode families and genera - an outline for soil ecologists. *J. Nematology*, 25(3), 315-331.
- Zavaleta, E. S., Shaw, M. R., Chiariello, N. R., Thomas, B. D., Cleland, E. E., Field, C. B., et al. (2003). Grassland responses to three years of elevated temperature, CO₂, precipitation, and N deposition. *Ecological Monographs*, 73(4), 585-604.

Table 17. Soil Chemistry at Field Site.

Soil chemistry parameters for all 25 plots (0 to 10 cm) at Colorado Plateau field site near Moab, UT (J Belnap, unpublished data).

<u>Variable</u>	<u>Mean</u>	<u>Stdev (n = 25)</u>
pH	8.11	0.126
OM (%)	0.38	0.224
CaCO ₃ (%)	3.07	0.761
CEC (cmol _c kg ⁻¹)	6.35	1.127
Ca (mg kg ⁻¹)	2688	362.4
Cu (mg kg ⁻¹)	0.41	0.083
Fe (mg kg ⁻¹)	2.53	0.491
K (mg kg ⁻¹)	151	69.9
Mg (mg kg ⁻¹)	148	44.8
Mn (mg kg ⁻¹)	4.45	0.742
Na (mg kg ⁻¹)	52.4	13.6
P (mg kg ⁻¹)	6.68	2.83
Zn (mg kg ⁻¹)	0.18	0.090
N (mg kg ⁻¹)	46.00	37.04

Table 18. Food Web Model Parameters.

Parameters used for construction of nitrogen cycling food web model from Hunt et al. (1987) unless noted otherwise.

Functional Group	C:N Ratio	Generation Time (days)	Assimilation Efficiency (e_{ass})	Production Efficiency (e_{prod})
Zoophagous mites	8	21.74	0.6	0.35
Microphytophagous mites	8	21.74	0.5	0.35
Oribatid mites	8	33.33	0.5	0.35
Collembolans	8	21.74	0.5	0.35
Tardigrades	8	21.74	0.5	0.35
Omnivore nematodes	10	50.0 ^a	0.5	0.37
Fungivore nematodes	10	20.83	0.38	0.37
Herbivore nematodes	10	37.04	0.25	0.37
Bacterivore nematodes	10	14.93	0.6	0.37
Amoebae	7	2 ^b	0.95	0.40
Ciliates	7	2 ^b	0.95	0.40
Flagellates	7	2 ^b	0.95	0.40
Bacteria	4	.	.	.
Fungi	10	.	.	.
Cyanobacteria	5	.	.	.
Roots	7	.	.	.

^a (Wood, 1973b).

^b (Darby et al., 2006).

Table 19. Analysis of Microfauna from Field Experiment.

(A) F-values from analysis of variance on \log_{10} to transformed biomass ($\mu\text{g kg}^{-1}$) of amoebae, flagellates, ciliates, and nematodes from the field experiment, followed by (B) comparison of least-squares means of overall log-transformed abundance throughout the experiment.

A)	<u>Effect</u>	<u>df</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Heat [†]	1,63	0.00	0.09	0.04	1.77
	Water [†]	1,63	0.80	0.53	0.23	0.02
	Heat*Water [†]	1,63	4.97	0.51	0.98	2.47
	Sampling	3,63	15.70***	14.09***	27.86***	2.84*
	Heat*Sampling	3,63	0.60	0.66	3.29*	0.07
	Water*Sampling	3,63	0.10	2.83	4.21**	1.18
	Heat*Water*Sampling	3,63	2.70	0.89	0.97	1.94
B)	<u>Sampling</u>		<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Spring '06		2.45 ^C	1.16 ^C	1.98 ^B	2.73 ^{AB}
	Fall '06		2.68 ^B	1.16 ^C	2.48 ^A	2.66 ^B
	Spring '07		2.74 ^B	1.50 ^A	2.50 ^A	2.78 ^A
	Fall '07		2.99 ^A	1.33 ^B	2.55 ^A	2.75 ^A
	(SE)		0.056	0.046	0.051	0.042

[†]No significant effect indicates no artificial pre-existing effect.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column) indicate contrasting means within an effect ($p < 0.05$).

Table 20. Nematode Genera Found at Field Site.

The following genera were found at the field site from 2006 to 2007. Families are divided into eight functional guilds for descriptive purposes on the basis of feeding morphology and life history traits (Yeates et al., 1993).

<u>Guild</u>	<u>Family</u>	<u>Genus</u>
<u>Omnivores: (fine-stylet bearing microphytophagous tylenchids)</u>		
	Tylenchidae	<i>Tylenchus</i>
		<i>Filenchus</i>
		<i>Coslenchus</i>
	Anguinidae	<i>Ditylenchus</i>
	Aphelenchidae	<i>Aphelenchus</i>
	Aphelenchoididae	<i>Aphelenchoides</i>
		<i>Anomyctus</i>
<u>Herbivores: (stomatostylet-bearing tylenchids)</u>		
	Paratylenchidae	<i>Paratylenchus</i>
	Pratylenchidae	<i>Pratylenchus</i>
	Criconematidae	<i>Criconemella</i>
	Belonolaimidae	<i>Tylenchorhynchus</i>
		<i>Merlinius</i>
<u>Bacterivores: (enrichment-to-mesic rhabditids)</u>		
	Panagrolaimidae	<i>Panagrolaimus</i>
	Rhabdolaimidae	<i>Rhabdolaimus</i>
	Plectidae	<i>Plectus</i>
<u>Bacterivores: (basal-type cephalobids)</u>		
	Cephalobidae	<i>Acrobeles</i>
		<i>Acrobeloides</i>
		<i>Cervidellus</i>
		<i>Chiloplacus</i>
		<i>Heterocephalobus</i>

	<i>Nothacrobeles</i>
	<i>Paracrobeles</i>
	<i>Placodira</i>
	<i>Stegelleta</i>
	<i>Stegelletina</i>
	<i>Acromoldavicus</i>
Ostellidae	<i>Drilocephalobus</i>
<u>Herbivores: (odontostyle-bearing dorylaims)</u>	
Longidoridae	<i>Xiphinema</i>
Nordiidae	<i>Longidorella</i>
	<i>Pungentus</i>
<u>Omnivores: (odontostyle-bearing dorylaims)</u>	
Qudsianematidae	<i>Discolaimium</i>
	<i>Discolaimoides</i>
	<i>Eudorylaimus</i>
	<i>Microdorylaimus</i>
	<i>Ecumenicus</i>
	<i>Lordellonema</i>
Aporcelaimidae	<i>Aporcelaimellus</i>
<u>Predators: (dorylaims with armed stoma)</u>	
Nygolaimidae	<i>Nygolaimus</i>
Carcharolaimidae	<i>Carcharolaimus</i>
Belondiridae	<i>Axonchium</i>
	<i>Dorylaimellus</i>
Leptolaimidae	<i>Tylencholaimellus</i>
Tylencholaimidae	<i>Tylencholaimus</i>
Campydoridae	<i>Campydora</i>

Table 21. Analysis of Nematode Composition from Field Experiment.

(A) F-values from analysis of variance on nematode diversity at the genus level and bacterivores (arcsine of square-root transformed proportions) from the field experiment, followed by (B) comparison of least-squares means of overall diversity and the proportion of nematodes that are bacterivores (arcsine of square-root transformed).

A)	<u>Effect</u>	<u>df</u>	<u>Diversity</u>	<u>Bacterivores</u>
	Heat [†]	1,63	0.14	0.34
	Water [†]	1,63	0.66	0.04
	Heat*Water [†]	1,63	1.19	0.03
	Sampling	3,63	18.27***	8.71***
	Heat*Sampling	3,63	0.48	0.27
	Water*Sampling	3,63	0.13	0.58
	Heat*Water*Sampling	3,63	2.54	0.31
B)	<u>Sampling</u>		<u>Diversity</u>	<u>Bacterivores</u>
	Spring '06		2.23 ^A	0.87 ^C
	Fall '06		1.54 ^C	1.05 ^A
	Spring '07		1.88 ^B	0.95 ^B
	Fall '07		1.87 ^B	1.05 ^A
	(SE)		0.077	0.035

[†]No significant effect indicates no artificial pre-existing effect.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column) indicate contrasting means within an effect ($p < 0.05$).

Table 22. Faunal Contributions to Nitrogen Cycling.

Nitrogen cycling results from soil food web model (adapted from Hunt et al., 1987), including standing biomass and nitrogen contributions to inorganic substrates and organic substrates through feces and death (all units in mg N). Death of amoebae, ciliates, and flagellates was computed as for other organisms (inverse of generation time) but are presented in parentheses because they are thought to not die naturally but rather continue to divide. Thus, contributions to substrate from death may be much less than modeled and limited to environmentally induced mortality rather than natural turnover.

Functional Group	Biomass (m ⁻²)	Inorganic (m ⁻² yr ⁻¹)	Feces (m ⁻² yr ⁻¹)	Death (m ⁻² yr ⁻¹)
Zoophagous	35.6	0.76	0.90	0.60
Microphytophagous	88.4	1.99	3.56	1.57
Oribatid	33.1	0.50	0.89	0.39
Collembolans	1070.7	24.14	43.10	18.97
Tardigrades	9.6	0.03	0.06	0.03
Dorylaim omnivore nemas	88.6	0.19	0.30	0.11
Tylenchid omnivore nemas	541.6	2.21	5.71	1.30
Herbivore nemas	2.7	0.007	0.03	0.004
Bacterivore nemas	1861.8	33.25	26.08	5.87
Amoebae	2406.7	161.14	11.01	(48.13)
Ciliates	1185.1	79.95	5.47	(23.88)
Flagellates	98.6	6.90	0.47	(2.06)
Total (mg N)	7422.5	311.08	97.61	102.91

Table 23. Predation Rates of Omnivorous Desert Soil Fauna.

Estimated predation rates ($\text{mg N m}^{-2} \text{ yr}^{-1}$) of omnivorous desert soil fauna (PF = plant feeding nematodes, FF = fungivorous tylenchid nematodes, OP = omnivorous dorylain nematodes, TAR = tardigrades, CO = collembolans, OR = oribatid mites, MI = microphytophagous prostigmatid mites, ZO = zoophagous prostigmatid mites. Values were modeled according the approach of Hunt et al. (1987).

Consumer →	ZO	MI	OR	CO	TAR	OP	FF	PF
Prey ↓								
MI	0.082							
OR	0.031							
CO	0.99							
TARD	0.0089							
OP	0.041	0.00088			<0.00001			
FF	0.25	0.0054			0.00001	0.00045		
PF	0.0013	0.00003			<0.00001	<0.00001		
BF	0.86	0.018			0.00003	0.0016		
FUNG		0.14	0.35	1.7	0.0024	0.012	1.5	
CYANO		7.0	1.7	85	0.12	0.60	7.7	
ROOTS								0.046

Table 24. Predation Rates of Bacterivorous Desert Soil Fauna.

Estimated predation rates ($\text{mg N m}^{-2} \text{ yr}^{-1}$) of bacterivorous desert soil fauna (BF = bacterivorous nematodes, AM = amoebae, CIL = ciliates, FLAG = flagellates). Values were modeled according the approach of Hunt et al. (1987).

Consumers →	BF	AM	CIL	FLAG
Prey ↓				
CILIATES		0.18		
FLAGELLATES		0.015	0.074	
BACTERIA	65	220	110	9.4

Table 25. Intra-Annual Variability of Microfauna Populations and Composition.

Summary table by sampling intervals (columns) indicating increases (↑), decreases (↓), or no changes (=) in populations and community indices.

Parameter	Spring '06	--	Fall '06	--	Spring '07	--	Fall '07
Cumulative Growth Potential	-3.33		+0.88		-3.78		
Nematodes	=		↑		=		
Amoebae	↑		=		↑		
Flagellates	=		↑		↓		
Ciliates	↑		=		=		
Nematode Diversity	↓		↑		=		
Bacterivorous Nematodes	↑		↓		↑		

Figure 16. Data to Estimate Growth Potential Index.

Data used to estimate growth potential index parameters. Top: Temperature-dependant protozoan growth rates (solid lines, from Darby et al., 2006) overlaid with growth rate function (dashed lines). Bottom: Moisture-dependant active nematode proportions (points, from Freckman and Mankau 1986) overlaid with proportion active function (solid line).

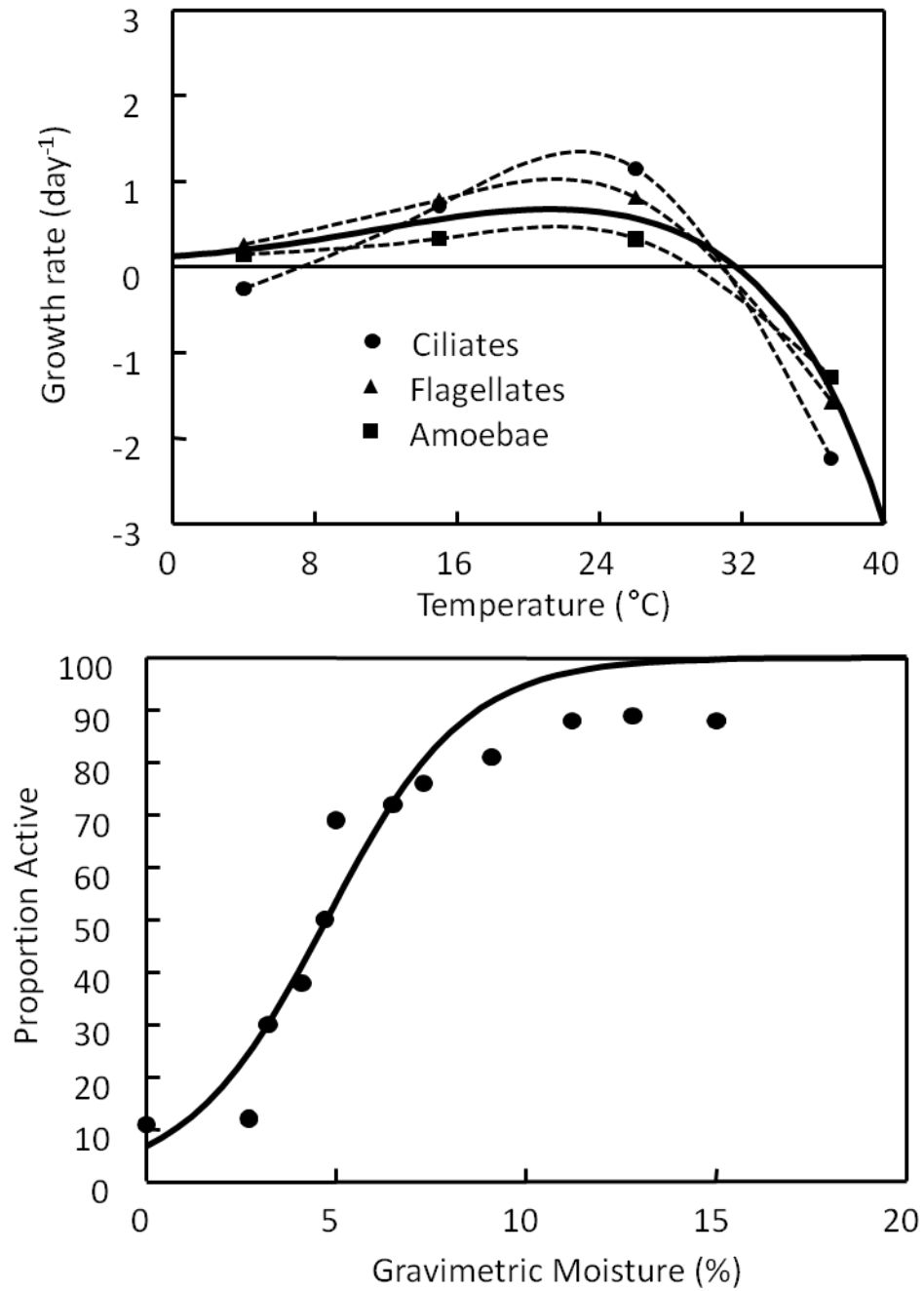


Figure 17. Observed Climate at Field Site.

Observed mean monthly temperature (solid lines) and observed monthly precipitation (solid bars) collected from field site, and historical mean monthly temperature (dashed lines) and historical median monthly precipitation (dashed bars) were collected from 1971-2000 monthly records at Moab, UT (www.ncdc.noaa.gov)

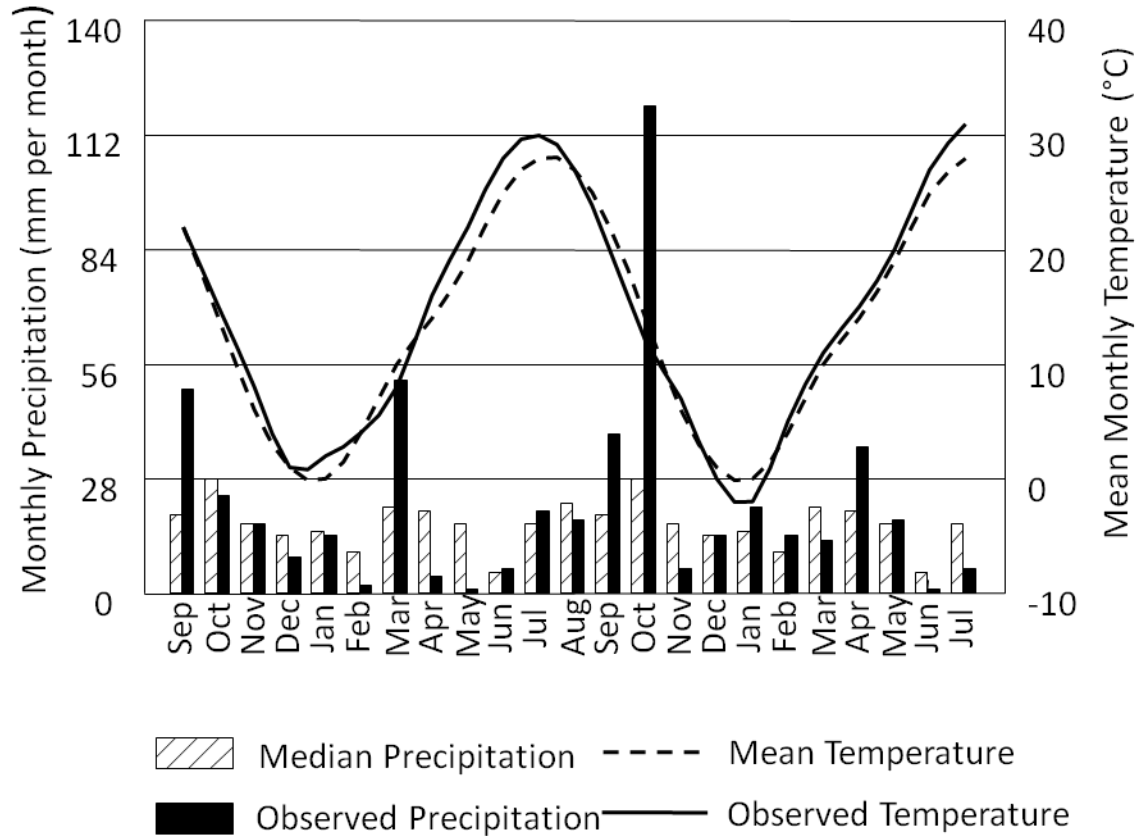


Figure 18. Seasonal Lamp Performance.

Mean difference of warmed plots from control plots (year 2006) smoothed with non-parametric local regression procedure using a smoothing parameter of 0.25. Graphs represent seasonal differences at 5 cm depth (top graph) and 15 cm depth (bottom graph) at 3:00 am and 15:00 pm (as labeled).

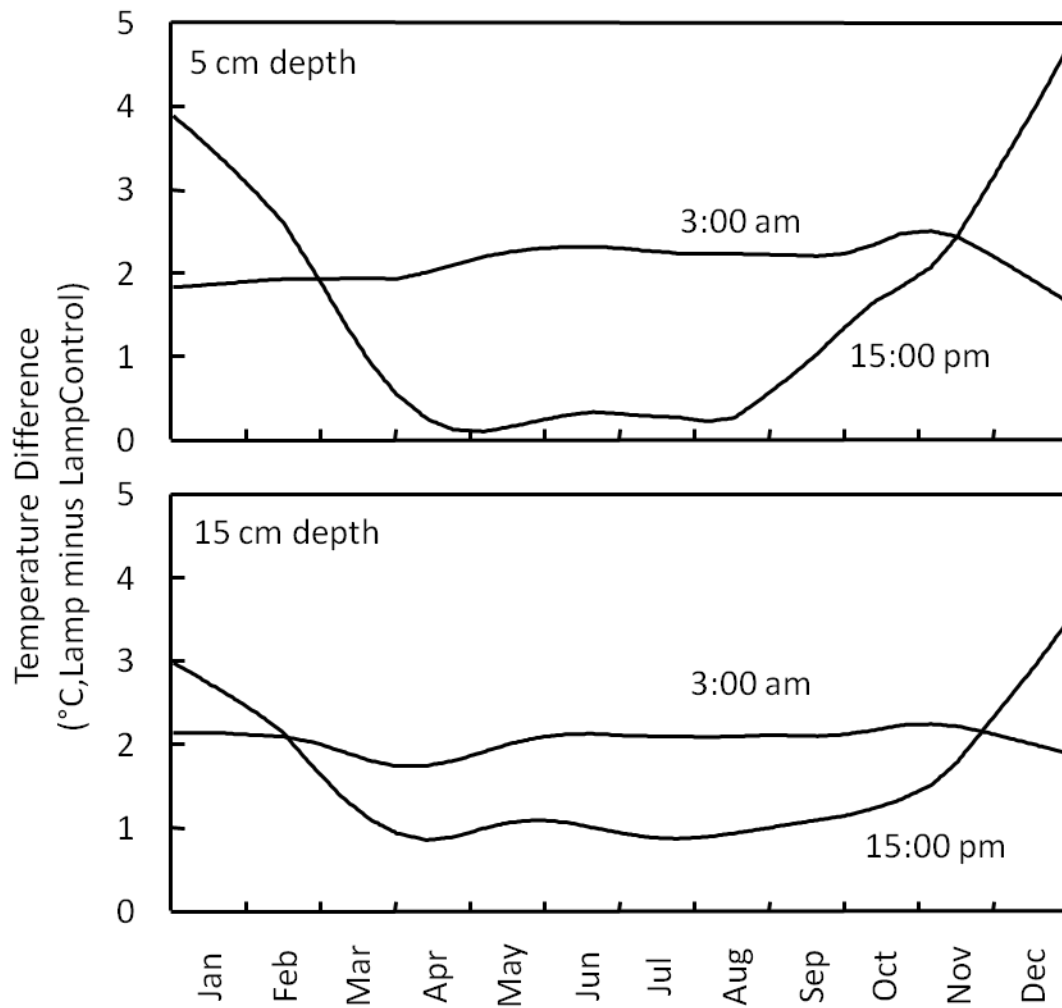


Figure 19. Diurnal Lamp Performance.

Mean difference of warmed plots from control plots (year 2006) smoothed with a non-parametric local regression procedure using a smoothing parameter of 0.25. Graphs represent diurnal differences at 5 cm depth (top graph) and 15 cm depth (bottom graph) during January and July of 2006 (as labeled).

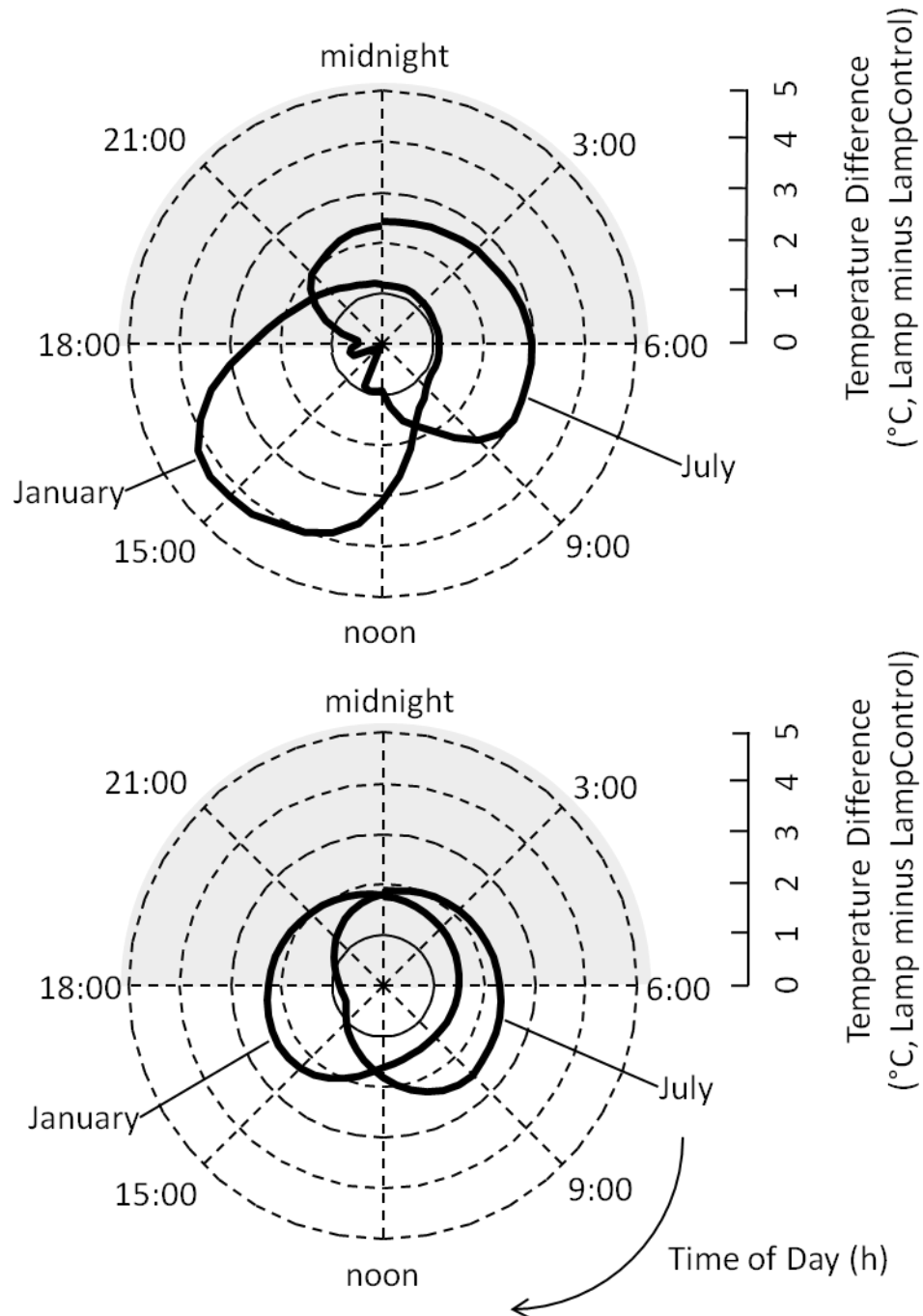


Figure 20. Growth of Microfauna through Two Experimental Years.

Abundance of nematodes and protozoa through four sampling events (note \log_{10} scale), and bacterivorous nematodes as a proportion of all nematodes. Values represent geometric means: back-transformed least-squared means of log-transformed abundance.

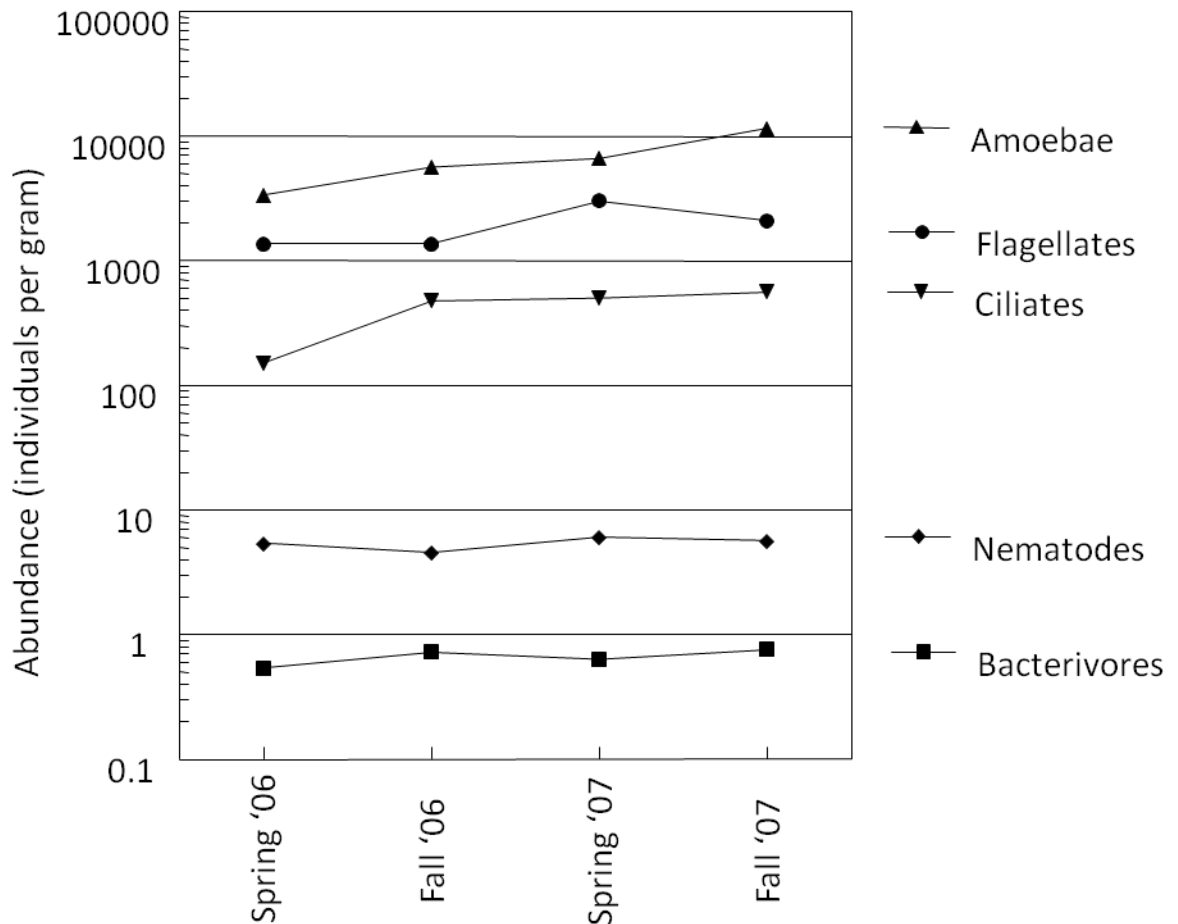


Figure 21. Microfauna Biomass under Elevated Temperature and Precipitation.

Biomass of (A) amoebae, (B) nematodes, (C) flagellates, and (D) ciliates through four sampling events from four treatments, ambient temperature and precipitation ('Control'), elevated temperature ('+Temp'), elevated precipitation ('+Precip'), or elevated temperature and precipitation ('+Temp+Precip'). Note \log_{10} scale, biomass converted from abundance by assuming 0.10 μg per nematode, 0.0000848 μg per amoebae, 0.0000106 μg per flagellate, and 0.000636 μg per ciliate (Petersen and Luxton 1982, Griffiths et al., 1995).

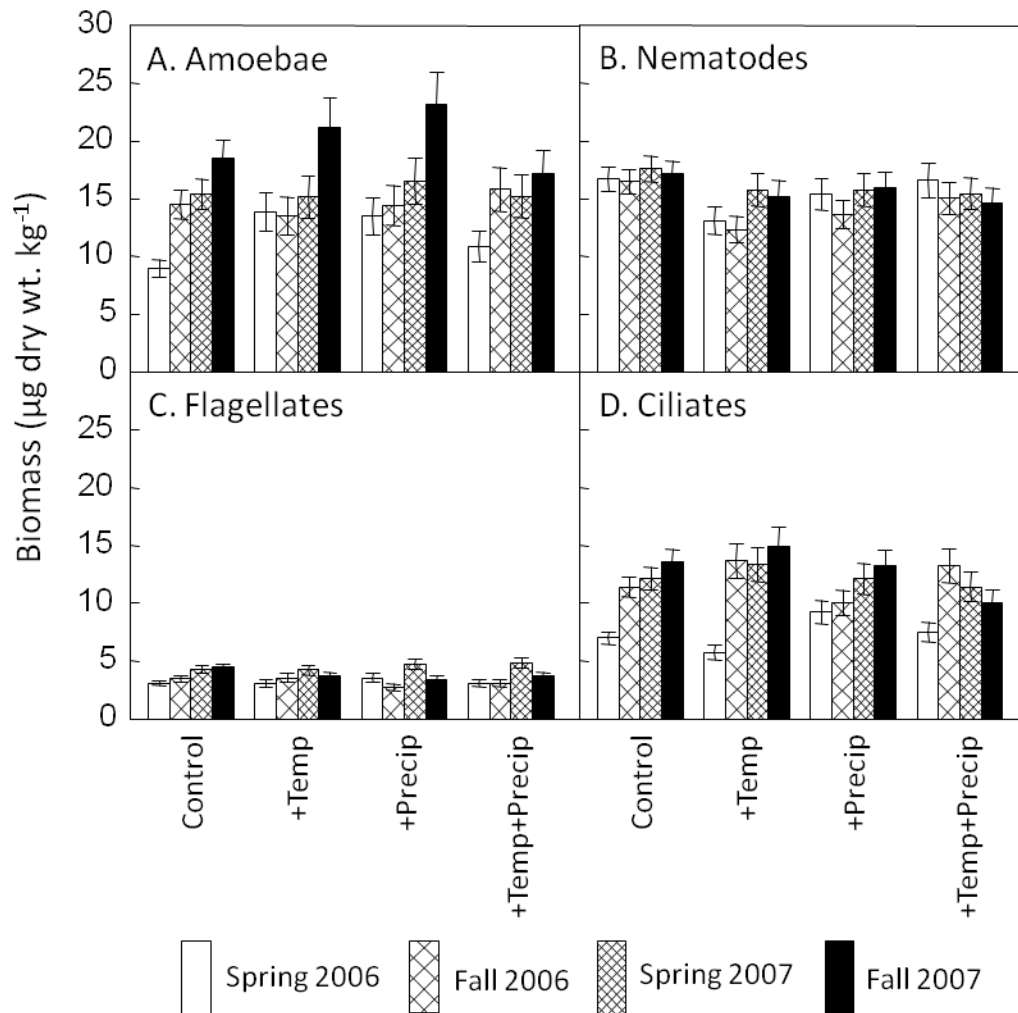
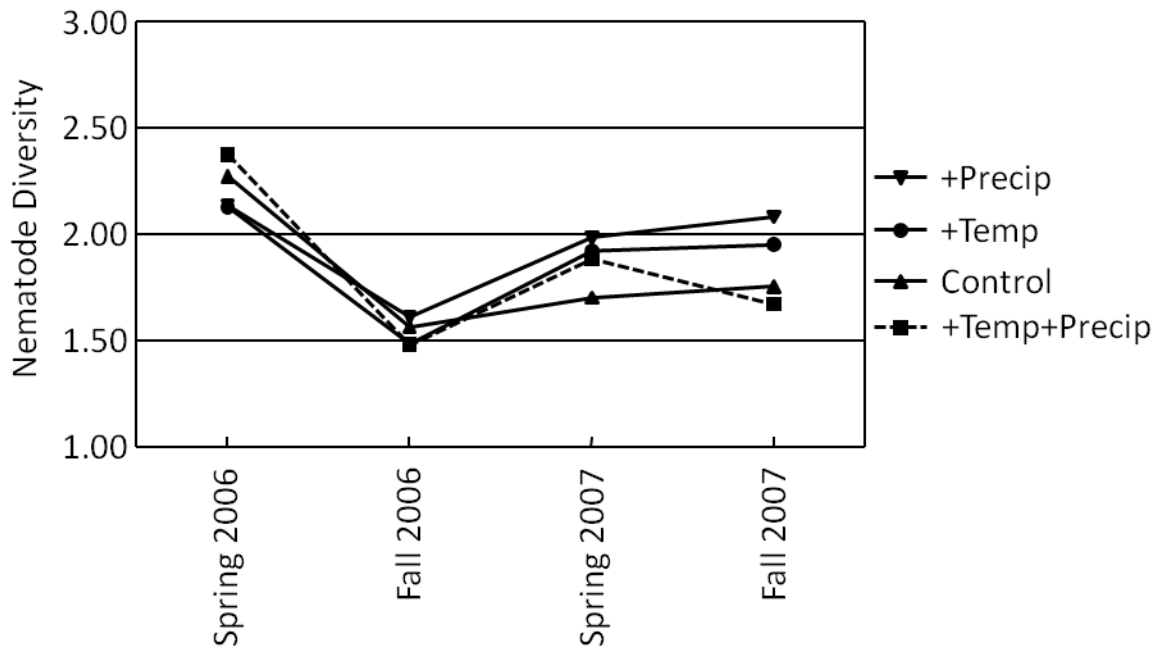


Figure 22. Nematode Diversity through Elevated Temperature and Precipitation.

Nematode genus diversity from field experiment from four treatments, ambient temperature and precipitation ('Control', triangles), elevated temperature ('+Temp', circles), elevated precipitation ('+Precip', upside-down triangle), or elevated temperature and precipitation ('+Temp+Precip' squares). Nematode genus diversity in plots receiving both elevated temperature and elevated precipitation declined more through summer 2006 and summer 2007 than other plots.



CHAPTER 6. INDIRECT EFFECTS OF ELEVATED CARBON DIOXIDE ON DESERT SOIL NEMATODES AND PROTOZOA.

Atmospheric carbon dioxide concentrations (CO_2) are rising and have the potential to increase photosynthesis, biomass, and water-use efficiency of some plants (Bazzaz, 1990). In desert habitats, elevated CO_2 has been linked with lower leaf litter quality (Billings et al., 2003) as well as the productivity and success of some invasive plants (Smith et al., 2000). Increased water-use efficiency was expected to benefit vegetation in dry regions more than moist temperate regions (Melillo et al., 1993). However, Housman et al. (2006) found that the enhanced vascular plant productivity from elevated CO_2 was restricted to years with abundant rainfall and that increased water-use efficiency did not help to increase productivity. Only the drought-deciduous shrub *Ambrosia* had significantly higher production under elevated CO_2 than ambient in three years of below-average rainfall. Nowak et al. (2004) found no difference in soil moisture content between ambient and elevated CO_2 in the top 20 to cm with measurements made at approximately 18-day intervals. Soil moisture in the top 50 to cm was also similar between ambient and elevated CO_2 for most (75 %) of the sampling dates, but when different, soil moisture was more frequently drier in the elevated CO_2 plots than the ambient CO_2 plots.

Microbial respiration depletes O_2 and raises CO_2 partial pressures upon rapid rewetting of dry soils (Fierer and Schimel, 2003) and the tortuosity of porous media

slows gaseous diffusion with the atmosphere. At the scale of individual soil pore spaces, carbon dioxide anesthetizes nematodes at a high threshold, estimated to be 10 to 40% CO₂ in water by Robinson and Heald (1991) for *Rotylenchulus reniformis*. Similarly, nematodes become motionless at low oxygen concentrations below a certain threshold (estimated at 0.8 ppm O₂, Robinson et al., 1981). However, carbon dioxide gradients also attract some nematodes at low concentrations (< 1-2% CO₂) (Robinson 1995). At an ecosystem scale, the most significant impact of elevated CO₂ on soil nematodes may be indirect from altered plant phenology. Plants benefiting from elevated CO₂ can increase belowground allocation of carbon and, consequently, are postulated to support greater nematode biomass of both herbivorous and microbivorous. Neher et al. (2004) tested this hypothesis in loblolly pine and sweet Gum plantations in eastern USA but found that elevated CO₂ decreased the total abundance of nematodes in both forest types. They concluded that the effect of elevated CO₂ on soil nematode communities is not likely a simple response to belowground carbon allocation. Alternatively, elevated CO₂ has the potential to increase water-use efficiency in plants that respond by reducing stomatal conductance. Nematode and protozoan activity is restricted to periods of adequate temperature and moisture, so elevated CO₂ could affect nematode community dynamics by altering soil rhizosphere hydrology. However, analysis of three grassland nematode herbivore communities to elevated CO₂, including two arid to semi-arid locations, found virtually no effect of elevated CO₂ on the abundance of herbivores (Ayres et al., 2008).

The objective of this study is to determine whether soil nematode and protozoan communities differed between field plots after receiving ambient versus elevated atmospheric CO₂ concentrations for eight years in the Mojave Desert. The study serves as a comparison to previous field tests to weigh the effects of elevated CO₂ relative to the effects of altered temperature and precipitation (Chapter 5).

Methods

The Nevada Desert FACE Facility (NDFF), located at the Nevada Test Site in southern Nevada, USA (36°49'N, 115°55'W, 965-970 m elevation), was designed to test the effect of elevated carbon dioxide on the desert vegetation and soil ecosystem. In April 2007, samples were collected from six rings, 25 m in diameter, surrounded by 32 vertical tubes that delivered ambient (360 µL L⁻¹, n = 3) or elevated (550 µL L⁻¹, n = 3) CO₂ (Jordan et al., 1999). From each ring, duplicate samples were collected from surface (0 to 0.5 cm) and subsurface (0 to 10 cm) soil from beneath three cover types: grass, shrub and interspace. In sum, 72 samples were collected for analysis (factorial combination of 2 CO₂, 3 rings, 3 cover types, 2 depths and 2 replicates).

Nematodes and protozoa were extracted and counted as described previously (Chapter 2) for all 72 samples but replicates were combined for nematode identifications resulting in 36 samples for nematode community composition indices. Three indices were computed to describe the nematode community composition: diversity (Shannon, 1948) was computed as $\sum p_i \cdot \ln(p_i)$, where p_i is the proportion of each genus i (n_i/N), richness was computed as the total number of genera found in each sample, and, the combined

maturity index (Bongers, 1990, as modified by Yeates, 1994) was computed as the weighted mean of colonizer-persistor values: $\sum p_i * cp_i$, where p_i is the proportion of each genus i and cp_i is the cp -value of genus i . Genera from families thought to represent faster-growing, colonizer-type individuals are assigned a low cp -value (1 or 2) and genera from families thought to represent slower-growing, persistor-type individuals are assigned a high cp -value (4 or 5). Consequently, low maturity values represent a community dominated by fast-growing, colonizer-type individuals while high maturity values represent a community with relatively more slow-growing, persistor-type individuals (Bongers 1990).

Hydrolysis of fluorescein-diacetate (FDA) was assayed as a proxy for microbial activity (as in Green et al., 2006, Aseri et al., 2006). At the initiation of the protozoan MPN dilutions, 5.0 ml of the first dilution (9 g soil in 80 ml sterile deionized water) was added to a sterile 45 ml plastic centrifuge tube and immediately frozen at -20 °C. At the time of analysis, the frozen samples in centrifuge tubes were thawed within 15 min in a 37 °C water bath. Immediately after thawing, 35 ml of 60 mM phosphate buffer (pH 7.6) was added to the sample tube, plus 0.5 ml of 3.6 mM FDA in acetone, and incubated at 35 °C for 1.5 h. Three times during the incubation (at 0, 30, and 60 min) the sample tubes were lightly shaken with three inversions to re-distribute in situ enzymes and the FDA substrate. Five minutes prior to the completion of the 1.5 h incubation, the sample tubes were centrifuged at 5,000 rev min⁻¹ for 5 min and the supernatant transferred to acrylic cuvettes and absorbance was measured on a spectrophotometer at 490 nm. Absorbance

was converted to concentration of fluorescein (hydrolyzed from FDA by in situ enzymes) and results are reported as $\mu\text{mol fluorescein g}^{-1} \text{ hr}^{-1}$ and not transformed prior to analysis.

Analysis

All dependant variables (\log_{10} to transformed amoebae, flagellate, ciliate, and nematode abundance, non-transformed nematode diversity, nematode richness, nematode maturity, FDA activity and \log_{10} to transformed gravimetric moisture) were analyzed with mixed model analysis of variance (PROC MIXED, SAS Institute, Inc., Cary, NC, USA) in a randomized complete block design. Carbon dioxide, cover type, and depth were used as fixed independent variables and ring and ring*cover type*depth as random variables. Multiple comparisons were made by Fisher's Least Significant Difference (LSD) Test: if treatment effects were found to be significant then means were compared by studentized *t*-tests, if treatment effects were not found to be significant then no means comparisons were made.

The nematode community composition at 0 to 10 cm was analyzed by direct gradient canonical correspondence analysis (CCA) to identify patterns of association between nematode community composition, vegetative cover type, and CO₂ treatment. The relative abundance of all genera were included as species variables and six treatment combinations were used as the environmental variables (factorial combination of 3 cover types and 2 CO₂ treatments). Monte Carlo permutations were employed to test the significance of the first axis and the full model, and the results are presented as a bi-plot showing the relative abundance of genera as points and the six treatment combinations as

environmental vectors. Vectors pointing in opposite directions represent treatment combinations with contrasting community composition, and points lying near an environmental vector represent a high relative abundance of the genus for that treatment combination.

Results

Overall, numbers of amoebae, flagellates, ciliates, and FDA hydrolysis were greatest at 0 to 0.5 cm, while diversity, richness, maturity, and water content were greatest at 0 to 10 cm (Table 26, Table 27, and Table 28). However, numbers of amoebae, flagellates, ciliates, nematodes, diversity, richness, water content and FDA hydrolysis were all greater adjacent to plants than in interspaces.

At the time of sampling, gravimetric moisture was greater under elevated (1.65 %) than ambient (1.47 %, $F_{1,36} = 4.59$, $p = 0.0389$) CO₂. However, CO₂ treatment was not a significant effect for the remaining biological parameters except in the context of an interaction with cover type or depth. Flagellates were more abundant beneath grass and shrub canopy than in interspaces under ambient CO₂, but were in equal abundance under elevated CO₂ (Figure 23). Although ciliates were more abundant at 0 to 0.5 cm than at 0 to 10 cm, the difference was greater under elevated than ambient CO₂ (Figure 24, $F_{1,36} = 3.89$, $p = 0.0562$). The interaction between CO₂ and cover type for flagellates or between CO₂ and depth for ciliates could not be explained with gravimetric moisture content. At the time of sampling, gravimetric moisture content was intermediate to grass and shrub cover under ambient CO₂ but was lower than either grass or shrub under elevated CO₂

(Figure 23). Similarly, the difference between 0 to 0.5 cm and 0 to 10 cm gravimetric moisture was greater under ambient than elevated CO₂ (Figure 24, with no significant interaction term, $F_{1,36} = 2.25$, $p = 0.1428$). Finally, maturity index values were low at 0 to 0.5 cm under both ambient and elevated CO₂ (1.95 and 1.96, respectively), but were greater in number at 0 to 10 cm under ambient (2.36) than elevated (2.18, $F_{1,20} = 4.46$, $p = 0.0474$) CO₂ concentrations.

Thirty-four genera were found at the Nevada FACE field site, with more overlap in genera found at a Colorado Plateau field site (Chapter 5) than a Chihuahuan Desert field site (Darby et al. 2007), especially in bacterivorous Cephelobidae (Table 29). Following multivariate ordination, eigenvalues from the CCA axis 1 (0.154, $p = 0.0240$) and axis 2 (0.104) explained 69.4% of the total species–environment variance (Figure 25). The species–environment correlation for axis 1 was 0.852 and for axis 2 was 0.862.

Discussion

Although the total abundance of amoebae, flagellates, ciliates, and nematodes were similar at elevated and ambient CO₂ after eight years of treatment, the most striking difference in soil microfaunal communities was 1) the interaction effects that elevated CO₂ and other environmental factors had on microfaunal communities, such as depth (for flagellates and nematode community maturity index) and vegetative cover (for ciliates), and 2) the composition of nematode genera between ambient and elevated CO₂ plots. Gravimetric moisture content at the time of sampling does not explain the interaction between CO₂ and cover type for flagellates or depth for ciliates. However, Nowak et al.

(2004) did find that elevated CO₂ plots were drier on occasion than ambient plots, but the measurements were made at coarse (18-day) intervals. I hypothesize that ciliates at 0 to 10 cm (less abundant in elevated than ambient CO₂ plots) and nematode maturity index values at 0 to 10 cm (lower values in elevated than ambient CO₂ plots) reflects a subtle but more rapid depletion of soil moisture from photosynthetic demand that restricts the growth of ciliates and longer-lived, slower-growing nematodes (especially omnivorous and predaceous dorylaims) after wetting events.

A second finding was that Maturity Index values were greater under ambient than elevated CO₂ concentrations at 0 to 10 cm depth, but not 0 to 0.5 cm. Upon further inspection, it appears that the most conspicuous difference between ambient and elevated CO₂ communities at 0 to 10 cm depth was a combination of the greater relative abundance of *Acromoldavicus mojavicus* under elevated CO₂ and the greater relative abundance of *Carcharolaimus*, *Aporcelaimellus*, *Ecumenicus*, and *Discolaimium* under ambient CO₂ (Figure 25). *Acromoldavicus mojavicus* is a relatively fast-growing bacterivorous nematode in the family Cephalobidae that is common and relatively unique to the Mojave Desert and Colorado Plateau (Baldwin et al., 2001). Also, *A. mojavicus* is one of the more desiccation tolerant cephalobes in the Colorado Plateau (Chapter 4), so the abundance of *A. mojavicus* in elevated CO₂ plots may reflect a tendency for elevated CO₂ plots to dry faster than ambient plots and adversely affect other nematodes more than *A. mojavicus*. Conversely, *Carcharolaimus*, *Aporcelaimellus*, *Ecumenicus*, and *Discolaimium* are common representatives of the larger-sized, slower-growing

omnivorous dorylaims of western United States and throughout the Great Basin (Thorne 1974b). All four genera have the potential to be predaceous on other nematodes, especially *Carcharolaimus*, *Aporcelaimellus*, and *Discolaimium*, while *Aporcelaimellus* has been observed frequently to feed on cyanobacteria (personal observation, and see Wood, 1973). I hypothesize that if the total water use demand from increased productivity offsets the water-use efficiency from elevated CO₂, decreased available moisture under ambient CO₂ could restrict the abundance of dorylaim-type predator-omnivores that tend to require more available water or humid spaces. A decline of potential facultative predators and a greater relative abundance of bacterivores could result in increased grazing pressure on bacteria or increased mineralization potential from the bacterivorous nematodes that are under less predation pressure by slower-growing predatory nematodes. Additional studies are needed to characterize the current nature of bacterial grazing. If bacteria are determined to be overgrazed, then increased grazing pressure could reduce the biomass of actively growing bacterial cells and reduce the production of exogenous enzymes that drive decomposition. However, if bacteria are determined to be undergrazed, then increased grazing pressure could potentially reduce the concentration of inactive, senescent cells, replaced by actively growing cells, and in fact accelerate the production of exogenous enzymes that drive decomposition.

In conclusion, I hypothesize that any effect of elevated CO₂ on the abundance of microfauna will be subtle and, in the desert, likely a result of soil hydrology, plant phenology and water use patterns. More research is needed on detailing what, if any,

competitive interactions are taking place and whether the microfauna inhabiting water films are partitioning their grazing activity to certain periods of time after wetting events.

References

- Aseri, G. K., & Tarafdar, J. C. (2006). Fluorescein diacetate: A potential biological indicator for arid soils. *Arid Land Research and Management*, 20(2), 87-99.
- Ayres, E., Wall, D. H., Simmons, B. L., Field, C. B., Milchunas, D. G., Morgan, J. A., et al. (2008). Belowground nematode herbivores are resistant to elevated atmospheric CO₂ concentrations in grassland ecosystems. *Soil Biology & Biochemistry*, 40(4), 978-985.
- Baldwin, J. G., De Ley, I. T., Mundo-Ocampo, M., De Ley, P., Nadler, S. A., & Gebre, M. (2001). *Acromoldavicus mojaviensis* n. sp. (Nematoda: Cephalobidae) from the Mojave Desert, California. *Nematology*, 3, 343-353.
- Bazzaz, F. A. (1990). The response of natural ecosystems to the rising global CO₂ levels. *Annual Review of Ecology and Systematics*, 21, 167-196.
- Billings, S. A., Schaeffer, S. M., & Evans, R. D. (2003). Nitrogen fixation by biological soil crusts and heterotrophic bacteria in an intact Mojave Desert ecosystem with elevated CO₂ and added soil carbon. *Soil Biology & Biochemistry*, 35(5), 643-649.
- Bongers, T. (1990). The maturity index: An ecological measure of environmental disturbance based on nematode species composition. *Oecologia*, 83, 14-19.
- Fierer, N., & Schimel, J. P. (2003). A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Science Society of America Journal*, 67(3), 798-805.
- Green, V. S., Stott, D. E., & Diack, M. (2006). Assay for fluorescein diacetate hydrolytic activity: Optimization for soil samples. *Soil Biology & Biochemistry*, 38(4), 693-701.
- Housman, D. C., Naumburg, E., Huxman, T. E., Charlet, T. N., Nowak, R. S., & Smith, S. D. (2006). Increases in desert shrub productivity under elevated carbon dioxide vary with water availability. *Ecosystems*, 9(3), 374-385.
- Jordan, D. N., Zitzer, S. F., Hendrey, G. R., Lewin, K. F., Nagy, J., Nowak, R. S., et al. (1999). Biotic, abiotic and performance aspects of the Nevada Desert Free-Air CO₂ enrichment (FACE) facility. *Global Change Biology*, 5(6), 659-668.
- Melillo, J. M., McGuire, A. D., Kicklighter, D. W., Moore, B., Vorosmarty, C. J., & Schloss, A. L. (1993). Global climate-change and terrestrial net primary production. *Nature*, 363(6426), 234-240.
- Neher, D. A., Weicht, T. R., Moorhead, D. L., & Sinsabaugh, R. L. (2004). Elevated CO₂ alters functional attributes of nematode communities in forest soils. *Functional Ecology*, 18(4), 584-591.

- Nowak, M. A., & Sigmund, K. (2004). Evolutionary dynamics of biological games. *Science*, 303(5659), 793-799.
- Robinson, A. F. (1995). Optimal release rates for attracting *Meloidogyne incognita*, *Rotylenchulus reniformis*, and other nematodes to carbon-dioxide in sand. *Journal of Nematology*, 27(1), 42-50.
- Robinson, A. F., & Heald, C. M. (1991). Carbon-dioxide and temperature-gradients in Baermann funnel extraction of *Rotylenchulus reniformis*. *Journal of Nematology*, 23(1), 28-38.
- Robinson, A. F., Orr, C. C., & Heintz, C. E. (1981). Effects of oxygen and temperature on the activity and survival of *Nothanguina phyllobia*. *Journal of Nematology*, 13(4), 528-535.
- Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, 27, 379-423, 623-656.
- Smith, S. D., Huxman, T. E., Zitzer, S. F., Charlet, T. N., Housman, D. C., Coleman, J. S., et al. (2000). Elevated CO₂ increases productivity and invasive species success in an arid ecosystem. *Nature*, 408(6808), 79-82.
- Thorne, G. (1974). *Nematodes of the Northern Great Plains. Part II. Dorylaimoidea, in part* (Nemata: Adenophorea). Brookings, SD: Tech. Bull. 41, Agricultural Experiment Station, South Dakota State University.
- Wood, F. H. (1973). Biology of *Aporcelaimellus* sp. (Nematoda: Aporcelaimidae). *Nematologica*, 19, 528-537.
- Yeates, G. W. (1994). Modification and qualification of the nematode maturity index. *Pedobiologia*, 38, 97-101.

Table 26. Analysis of Microfauna under Elevated Carbon Dioxide.

A) F-values from analysis of \log_{10} transformed abundance of amoebae, flagellates, ciliates, and nematodes from the field experiment, followed by B) means comparisons of effect means for cover type on \log_{10} transformed abundance, and C) means comparisons of effect means for \log_{10} transformed abundance.

A)	<u>Effect</u>	<u>df</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Treatment	1,36	1.17	0.03	3.08	0.52
	Cover	2,20	7.22**	7.10**	7.51**	7.54**
	Treatment*Cover	2,36	0.56	3.92*	0.42	0.10
	Depth	1,20	47.38***	81.51***	74.31***	0.05
	Treatment*Depth	1,36	0.63	1.86	3.89	0.57
	Cover*Depth	2,20	0.00	0.16	2.03	6.93**
	Treatment*Cover*Depth	2,36	0.47	0.22	0.07	2.63

B)	<u>Cover</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Grass	3.79 ^A	3.40 ^A	2.87 ^A	0.45 ^A
	Interspace	3.56 ^B	3.16 ^B	2.60 ^B	0.32 ^A
	Shrub	3.85 ^A	3.44 ^A	2.92 ^A	0.64 ^B
	(SE)	0.058	0.057	0.064	0.060

C)	<u>Depth</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Surface (0 to 0.5 cm)	3.96 ^A	3.63 ^A	3.11 ^A	0.46
	Deep (0 to 10 cm)	3.50 ^B	3.04 ^B	2.48 ^B	0.48
	(SE)	0.047	0.047	0.052	0.049

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column)

indicate contrasting means within an effect ($p < 0.05$). Means without a letter indicate a non-significant effect and no comparisons were made.

Table 27. Analysis of Nematode Communities under Elevated Carbon Dioxide.

A) F-values from analysis of variance on non-transformed genus diversity, richness, and maturity from the field experiment, followed by B) means comparisons of effect means for cover type on non-transformed index values, and C) means comparisons of effect means for non-transformed index values.

A)	<u>Effect</u>	<u>df</u>	<u>Diversity</u>	<u>Richness</u>	<u>Maturity</u>
	Treatment	1,20	1.51	0.30	1.37
	Cover	2,20	5.29*	14.33**	0.65
	Treatment*Cover	2,20	0.62	0.076	0.05
	Depth	1,20	114.13***	243.62***	59.73***
	Treatment*Depth	1,20	0.63	0.23	4.46*
	Cover*Depth	2,20	1.09	9.48**	5.67*
	Treatment*Cover*Depth	2,20	0.30	4.97*	0.19

B)	<u>Cover</u>	<u>Diversity</u>	<u>Richness</u>	<u>Maturity</u>
	Grass	1.41 ^{AB}	10.67 ^B	2.11
	Interspace	1.23 ^A	8.58 ^A	2.08
	Shrub	1.63 ^B	12.33 ^C	2.14
	(SE)	0.104	0.612	0.047

C)	<u>Depth</u>	<u>Diversity</u>	<u>Richness</u>	<u>Maturity</u>
	Surface (0 to 0.5 cm)	0.89 ^A	6.06 ^A	1.96 ^A
	Deep (0 to 10 cm)	1.96 ^B	15.00 ^B	2.27 ^B
	(SE)	0.091	0.540	0.042

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column)

indicate contrasting means within an effect ($p < 0.05$). Means without a letter indicate a non-significant effect and no comparisons were made.

Table 28. Analysis of Soil Properties under Elevated Carbon Dioxide.

(A) F-values from analysis of variance on \log_{10} transformed gravimetric moisture content (%) and \log_{10} transformed FDA activity ($\text{nmol g}^{-1} \text{ hr}^{-1}$) from the field experiment, followed by B) means comparisons of effect means for cover type on \log_{10} transformed values, and C) means comparisons of effect means on \log_{10} transformed values.

A)	<u>Effect</u>	<u>df</u>	<u>Moisture</u>	<u>FDA activity</u>
	Treatment	1,36	4.59*	0.68
	Cover	2,20	8.21**	32.45***
	Treatment*Cover	2,36	3.68*	0.76
	Depth	1,20	428.01***	287.90***
	Treatment*Depth	1,36	2.25	0.92
	Cover*Depth	2,20	2.35	0.41
	Treatment*Cover*Depth	2,36	3.78*	1.43
B)	<u>Cover</u>		<u>Moisture</u>	<u>FDA activity</u>
	Grass		0.19 ^A	0.39 ^A
	Interspace		0.14 ^A	-0.04 ^B
	Shrub		0.25 ^B	0.48 ^A
	(SE)		0.020	0.055
C)	<u>Depth</u>		<u>Moisture</u>	<u>FDA activity</u>
	Surface (0 to 0.5 cm)		-0.05 ^A	0.75 ^A
	Deep (0 to 10 cm)		0.44 ^B	-0.20 ^B
	(SE)		0.017	0.047

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column)

indicate contrasting means within an effect ($p < 0.05$). Means without a letter indicate a non-significant effect and no comparisons were made.

Table 29. Cross-Desert Comparison of Nematode Genera.

Comparative incidence of genera at depths of 0 to 10 cm from three desert locations with similar sampling effort: Mojave Desert, Nevada (Spring 2007), Colorado Plateau, Utah (Spring 2007, Chihuahuan Desert, New Mexico (Fall 2002).

Guild	Genus	Mojave Desert	Colorado Plateau	Chihuahuan Desert
<u>Omnivores: (fine-stylet bearing microphytophagous tylenchids)</u>				
	<i>Anomyctus</i>		X	
	<i>Aphelenchoides</i>	X	X	X
	<i>Aphelenchus</i>	X	X	X
	<i>Coslenchus</i>	X		
	<i>Ditylenchus</i>	X	X	X
	<i>Filenchus</i>	X	X	X
	<i>Subanguina</i>	X		
	<i>Tylenchus</i>	X	X	
<u>Herbivores: (stomatostylet-bearing tylenchids)</u>				
	<i>Merlinius</i>	X		
	<i>Paratylenchus</i>		X	X
	<i>Pratylenchus</i>		X	X
	<i>Tylenchorhynchus</i>	X	X	X
<u>Bacterivores: (enrichment-type rhabditids)</u>				
	<i>Panagrolaimus</i>	X	X	
	<i>Rhabdolaimus</i>			
<u>Bacterivores: (aquic-type rhabditids)</u>				
	<i>Monhystera</i>	X		
	<i>Plectus</i>	X	X	X
<u>Bacterivores: (basal-type cephalobids)</u>				
	<i>Acrobeles</i>	X	X	X
	<i>Acrobeloides</i>	X	X	X
	<i>Acromoldavicus</i>	X	X	
	<i>Cervidellus</i>	X	X	X
	<i>Chiloplacus</i>	X	X	X
	<i>Drilocephalobus</i>	X	X	
	<i>Heterocephalobus</i>		X	
	<i>Nothacrobeles</i>	X	X	
	<i>Paracrobeles</i>	X		
	<i>Placodira</i>	X	X	
	<i>Stegelleta</i>	X	X	
	<i>Stegelletina</i>	X	X	X
<u>Herbivores: (odontostyle-bearing dorylaims)</u>				
	<i>Axonchium</i>	X	X	X

<i>Dorylaimellus</i>				X
<i>Longidorella</i>	X			
<u>Omnivores: (odontostyle-bearing dorylaims)</u>				
<i>Aporcelaimellus</i>	X	X		
<i>Aporcelaimium</i>				X
<i>Discolaimium</i>	X	X		
<i>Ecumenicus</i>	X	X		
<i>Eudorylaimus</i>	X	X		X
<i>Lordellonema</i>		X		
<i>Microdorylaimus</i>	X	X		
<i>Thonus</i>				X
<i>Tylencholaimellus</i>	X	X		
<i>Tylencholaimus</i>		X		X
<u>Predators: (dorylaims with armed stoma)</u>				
<i>Carcharolaimus</i>	X	X		
<i>Nygolaimus</i>	X	X		

Figure 23. Flagellates and Water Content under Elevated Carbon Dioxide.

Values represent flagellates abundance (numbers g^{-1} , top graph) and gravimetric moisture (% , bottom graph) under ambient (dashed fill) or elevated CO_2 (solid fill) for grass, interspace, and shrub cover (as labeled).

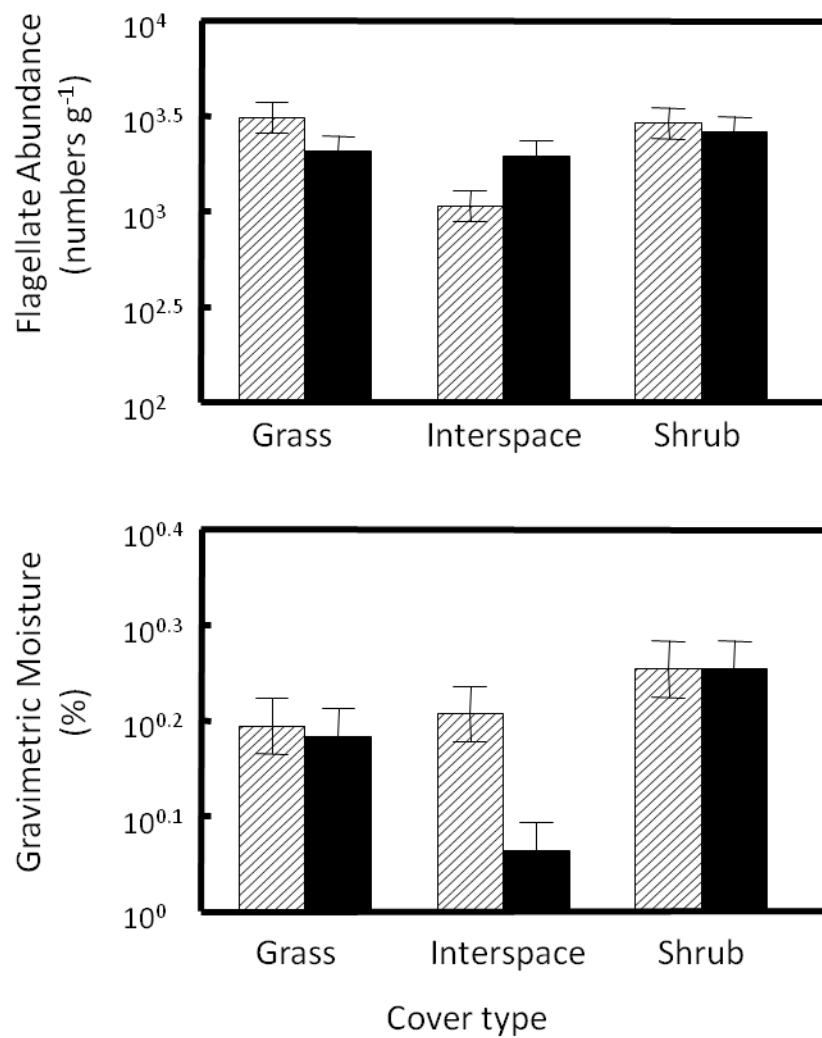


Figure 24. Ciliates and Water Content under Elevated Carbon Dioxide.

Values represent ciliate abundance (numbers g^{-1} , top graph) and gravimetric moisture (% , bottom graph) under ambient (dashed fill) or elevated CO_2 (solid fill) for 0 to 0.5 cm and 0 to 10 cm depth.

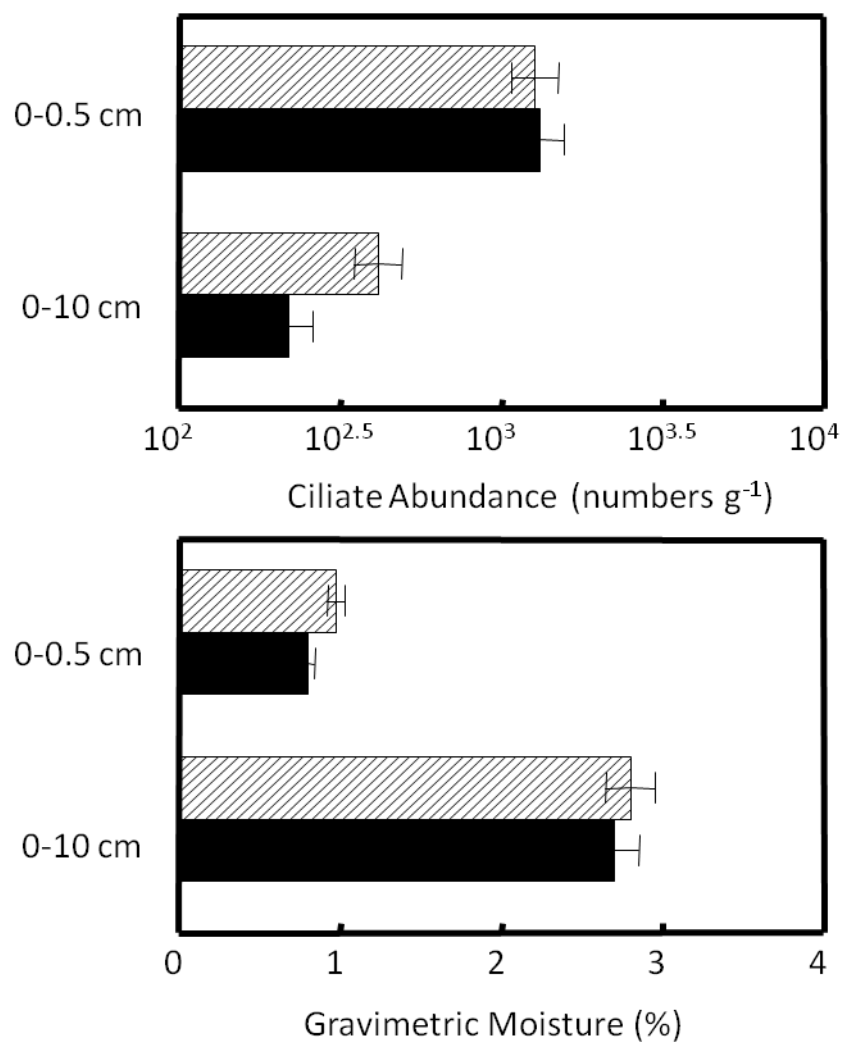
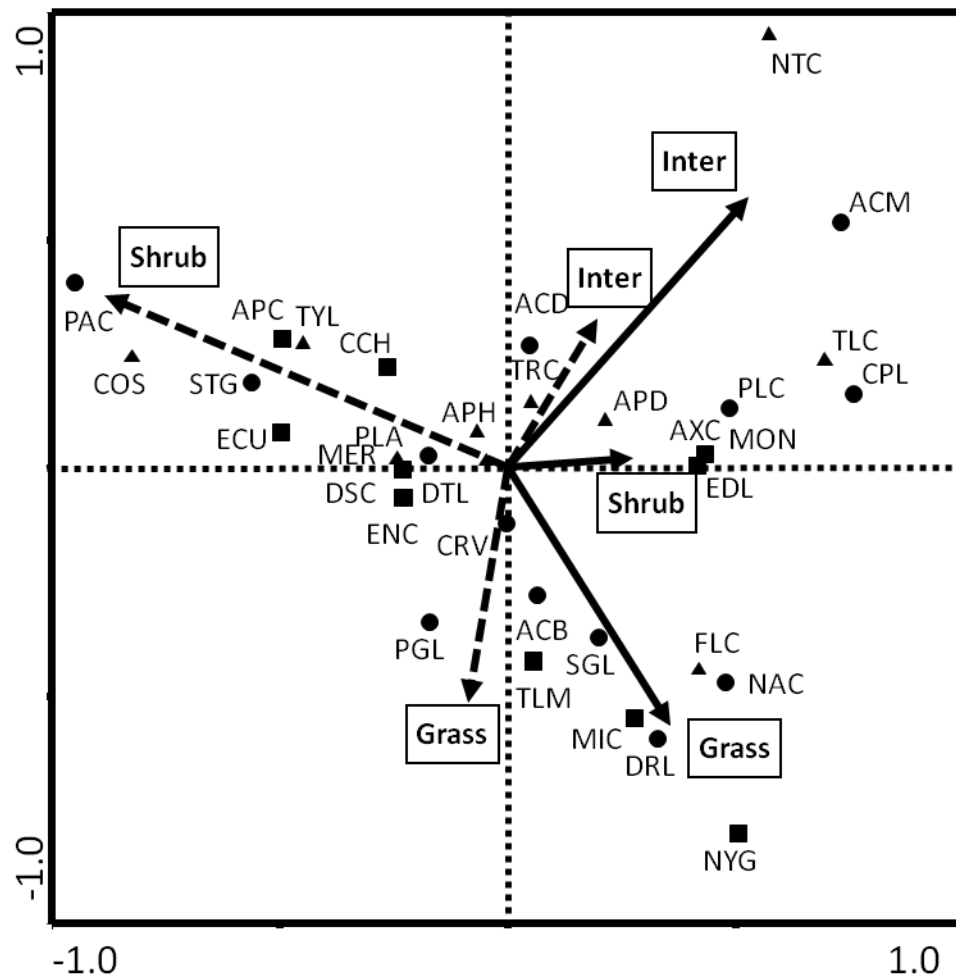


Figure 25. Nematode Community Composition under Elevated Carbon Dioxide.

Canonical Correspondence Analysis biplot of nematode genera. Environment vectors represent ambient (dashed vectors) versus elevated (solid vectors) CO₂ rings and three cover types, grass, shrub, and interspaces (as labeled). Points represent relative abundance of tylenchid piercers (triangles), dorylaimid piercers (squares), and bacterivores (circles): ACB, *Acrobeles*; ACD, *Acrobeloides*; ACM, *Acromoldavicus*; APC, *Aporcelaimellus*; APD, *Aphelenchoides*; APH, *Aphelenchus*; AXC, *Axonchium*; CCH, *Carcharolaimus*; COS, *Coslenchus*; CPL, *Chiloplacus*; CRV, *Cervidellus*; DRL, *Drilocephalobus*; DSC, *Discolaimium*; DTL, *Ditylenchus*; ECU, *Ecumenicus*; EDL, *Eudorylaimus*; ENC, *Enchodellus*; FLC, *Filenchus*; MER, *Merlinius*; MIC, *Microdorylaimus*; MON, *Monhystera*; NAC, *Nothacrobeles*; NYG, *Nygolaimus*; PAC, *Paracrobeles*; PGL, *Panagralaimus*; PLA, *Placodira*; PLC, *Plectus*; SGL, *Stegelletina*; STG, *Stegelletta*; SUB, *Subanguina*; TLC, *Tylenchus*; TLM, *Tylencholaimus*; TRC, *Tylenchorhyncus*; TYL, unidentified tylench.



CHAPTER 7. INTERACTIONS BETWEEN DESERT NEMATODES AND PROTOZOA

Bacterivorous nematodes and protozoa are predicted to contribute a significant portion of the nitrogen mineralization from fauna in a shortgrass prairie (Hunt et al., 1987), desert soil food web (Chapter 5), and other natural and agricultural systems (de Ruiter et al., 1993). Unfortunately, the biology and life history traits of nematodes and amoebae from desert soils make testing the model predictions difficult without artificial alterations to the experimental environment. Initial populations of microfauna are difficult to control in desert soil, without adverse confounding effects. Desert nematodes and protozoa rapidly increase in abundance following re-wetting if the soil is kept moist for a long period of time (>1 wk). Most empirical studies to test the relative role of fauna in soil nitrogen cycling take one of several approaches, 1) correlate changes in mineralization rates to changes in faunal abundance (Saetre and Stark, 2005), or 2) create treatments of presence and absence of one or more groups either by a) selective defaunation (Ingham et al., 1989; Beare et al., 1992), or b) general defaunation and selective re-inoculation (Cole et al., 2004). Biocides are semi-selective at best and may confound the experimental system (Ingham and Coleman, 1984). Furthermore, many studies address only one of several faunal groups while other groups are eliminated from the experimental system during defaunation, ignored and not measured, or measured but not incorporated explicitly into the design and interpretation. There is a need to develop

experimental conditions to examine the relative role that both nematodes and protozoa play in nitrogen cycling while maintaining controlled population sizes and minimizing adverse experimental artifacts.

Most species of desert nematodes and protozoa feed on bacteria and other small particles: by phagocytosis for protozoa and by ingestion through an unarmed buccal cavity for nematodes. Among all bacterivorous nematodes, the family Diplogasteridae contains several fast-growing predaceous genera that have been observed to feed on both amoebae and bacteria in addition to other nematodes. Using a representative diplogasterid, *Mesodiplogaster*, and amoebae, *Acanthamoebae*, Anderson et al. (1978) and Elliott et al. (1980) found greater proportional increase of nematode growth in the presence of amoebae than without amoebae, particularly in fine textured soils. They hypothesized that amoebae support nematode growth in this simplified food web by accessing bacterial prey in smaller pore spaces that are inaccessible to nematodes and make that food available to nematodes as prey themselves.

However, diplogasterids are generally absent from the desert soil habitats of southwestern United States. Insufficient moisture and prey biomass limits the accumulation of the fast-growing Diplogasteridae, as well as Rhabditidae and Panagrolaimidae, known for enriched habitats. Instead, Cephalobidae is the most abundant and speciose family of bacterivorous nematodes in the desert. Although bacteria are thought to be their main source of food, cytotrophy on small flagellates and protozoan cysts and mycophagy on fungal spores is also possible. However, pore spaces in arid soil

could be more inaccessible for nematodes than amoebae and the biotic interactions between bacterivorous nematodes and amoebae in desert soils may differ from temperate soils. Predation may be reversed, with active amoebae preying on nematodes trapped in fine pore spaces, or offset by competition, with active amoebae consuming prey. Amoebae ($0.0848 \text{ ng dry weight ind}^{-1}$) are expected to be active in smaller pore spaces with less available moisture than nematodes ($0.1 \text{ } \mu\text{g dry weight ind}^{-1}$) due to their difference in size. Furthermore, bacterivorous amoebae phagocytize prey in pores smaller than their mean body diameter by extending flexible pseudopods. While some Cephalobidae have elongated labial probolae that may help the nematode to access prey in pores smaller than their anterior body diameter, bacterivorous nematodes are likely constrained to larger spaces. Experimental systems are lacking to further examine the relative balance of these biotic interactions and test for the existence of competition.

The objective of this study is to test an experimental design to examine the 1) biotic interactions between nematodes and protozoa, and 2) relative influence of both groups on nitrogen cycling. Two constraints influenced the experimental setup. First, only destructive methods are available for enumerating nematodes and protozoa. Therefore, effective blocking is necessary in order to initiate uniform populations and track populations through time. Secondly, wetting and drying is a fundamental part of the natural history of desert soil microfauna and reasonable schedules of wetting and drying are necessary. Two microcosm experiments are established to allow sustained but controlled growth of nematodes and protozoa. The approach to assess biotic interactions

is to alter the abundance of nematodes (45% addition in the first experiment and 45% reduction in the second experiment) and examine the growth of amoebae. If biotic interactions are dominated by competition then growth of amoebae will be slowed with the addition of nematodes or accelerated with the partial removal of nematodes. If biotic interactions are dominated by amoebae preying on nematodes, then growth of amoebae will be accelerated with the addition of nematodes or slowed with the partial removal of nematodes. Furthermore, if competition for prey biomass exists then nitrogen mineralization will be greater in the systems with greatest amoebae biomass growth.

Methods

Microcosm Construction

Both experiments used a sandy loam soil at the site of an on-going field experiment near Canyonlands National Park that is representative of a cool-desert soil that experiences periodic wetting and drying. In both experiments, 10 samples of soil, each about 2 kg, were collected from the field, air dried, and sieved gently (2 mm) to remove rocks. Each sample was homogenized gently without contact by rotation of the soil in a plastic beaker and split into four subsamples of about 200 g each that were contained in plastic open-top microcosms (in addition to a fifth 200 to g aliquot used for baseline measurements). Previous experiments showed that this procedure resulted in subsamples of similar nematode and protozoan communities, both quantitatively and qualitatively. Five of the samples (and their four subsamples) were incubated at 36 °C and the remaining five were incubated at 26 °C to simulate warm, fast drying conditions

at the surface and cool, slow drying conditions at depth, respectively. The four microcosms from each sample were assigned randomly to one of two destructive harvest dates (week 4 or 8), and to one of two nematode treatments (field abundance or altered abundance). The first wetting included an addition of 35 ml sterile de-ionized water that evaporated completely by the end of one week. Thereafter, the microcosms were wetted each week with additional 35 ml sterile DI water, an amount found previously to bring soil moisture near -10 kPa, and allowed to dry naturally throughout the week. All of the subsamples in microcosms were amended with 1 ml 75.6 mM labeled ^{15}N ammonium sulfate (98+ atom%) at the time of the first wetting to estimate gross mineralization rates by the pool-dilution approach (Myrold and Tiedje, 1986). The rapid loss of ammonium precluded extraction of ammonium for isotopic analysis and further estimation of gross mineralization rates. The first and second experiments differ only in their application of the treatment to alter nematode abundance:

Experiment 1. Nematode Addition

Nematodes from 440 g of each sample were extracted and counted to estimate baseline abundance. Twenty percent of this sample community was removed for identification and the remaining 80% was divided in two parts and each part assigned randomly to one of the two microcosms of that block determined to receive the inoculant. The inoculum was added to the top of the microcosm at the time of the first wetting with no attempt to ‘stir’ the nematode inoculate into the soil as previous experiments found this created nearly 100% mortality (Saetre and Stark 2005).

Experiment 2. Nematode Removal

Nematodes were partially defaunated by heat to reduce nematode abundance rather than increase nematode abundance. Half of the samples were heated at 65°C for 48 h prior to receiving their first wetting and isotope label. In previous experiments, heating was found to disproportionately reduce the abundance of nematodes more than protozoa.

Destructive Harvest and Analysis

At the assigned destructive harvest for both experiments, nematodes and protozoa were enumerated from 100 g and 9 g, respectively, from each microcosm using methods previously discussed (Chapter 2). With soil from each microcosm, inorganic nitrogen was extracted with 2M KCl in a 10:1 dilution of 10 g soil, filtered, and frozen at -20 °C until analysis. Nitrate and ammonium were assayed colorometrically with a flow-injection autoanalyzer (Lachat QuickChem AE, Hach Instruments, Loveland, CO) using Cd-reduction for nitrate and salicylate-nitroprusside for ammonium. Abundance of nematodes and protozoa (log-transformed prior to analysis) and inorganic nitrogen concentrations were analyzed statistically with a blocked mixed model ANOVA. Multiple comparisons were made by Fisher's Least Significant Difference (LSD) Test: if treatment effects were found to be significant then means were compared by studentized *t*-tests, otherwise no means comparisons were made.

Results

Experiment 1. Nematode Addition

Nematode abundance in the inoculated treatments after the first four weeks was approximately 140 to 145% field abundance, suggesting 50 to 55% mortality of the inoculated nematodes and an overall 40 to 45% inoculation rate above field abundance (Figure 26). All protozoa increased during the first month of the first experiment (Table 30). Amoebae populations increased more at field abundance of nematodes than with addition of nematodes (Figure 27). Both flagellates and ciliates populations increased more the first month at the lower (26 °C day, 16 °C night) than the higher incubation temperature (36 °C day, 26 °C night). Ammonium was depleted at both temperatures (Table 31, Figure 1), while nitrate accumulated in both temperatures at the rate of 257 $\mu\text{mol g}^{-1} \text{wk}^{-1}$ or 8.56 $\mu\text{mol g}^{-1} \text{d}^{-1}$, assuming constant linear accumulation.

Experiment 2. Nematode Removal

Heat-treated nematodes were 55 % the abundance of the non-heated control abundance, indicating an overall 45% nematode defaunation rate (Table 32, Figure 28). Amoebae and flagellate populations increased exponentially through the two months of the second experiment, regardless of pre-treatment (Table 32, Figure 29), at an instantaneous growth constant of 0.211 wk^{-1} and 0.308 wk^{-1} , respectively. Incubation temperature affected growth of ciliates but not amoebae or flagellates. Ciliates declined the first month when kept at 26 °C but nearly doubled after two months when kept at 36 °C. Ammonium was depleted at both temperatures (Table 33, Figure 28), while nitrate

accumulated in both temperatures at the rate of $234 \mu\text{mol g}^{-1} \text{wk}^{-1}$ or $7.8 \mu\text{mol g}^{-1} \text{d}^{-1}$, assuming constant linear accumulation.

Discussion

The first experiment provided evidence of competition between nematodes and amoebae, but not the second. Amoebae populations increased more the first month without than with added nematodes. However, this was not the case in the second experiment; amoebae grew at similar rates with or without the partial defaunation of nematodes by heat. Partial defaunation of nematodes for 48 h at 65°C was applied as an alternative approach to changing nematode but not protozoan abundance because elevated temperatures were believed to affect nematodes more negatively than protozoa (chapter 4). Surface microbiota experience similar extreme temperatures in the field, so partial defaunation by heat was expected to minimize potential artifacts of disturbance in comparison to alternatives (e.g., biocides). No protozoan groups were affected by the partial defaunation of nematodes, but ciliate populations increased more when incubated at 36°C than at 26°C . This is surprising because growth of these desert protozoa in log-phase is a unimodal function of temperature with maximum growth around 24°C and negative growth (encystment or mortality) beginning around 32°C . However, increase of protozoan populations in periodically wetting and drying environments is not a simple function of temperature. Even in liquid culture, extreme temperature events induce encystment of active protozoa and alter the subsequent growth of protozoa following excystment at moderate temperatures (Darby et al., 2006). Further work is needed to

examine the effect of extreme temperatures while dry, or of prolonged drought conditions, on protozoan growth following excystment.

Overall, the experimental setup worked to control populations in a realistically wetting and drying environment, but was not able to retain an ammonium pool sufficient to conduct isotopic analysis and quantification of gross mineralization rates. Ammonium-oxidizing bacteria are prevalent in these desert soils, especially within the top 1 cm (Johnson et al., 2005), so it is reasonable to hypothesize that in these two experiments any inorganic nitrogen that was produced as ammonium was oxidized to nitrate at or near the rate of production. Low numbers of denitrifying bacteria in these soils reflect minimal denitrification rates relative to other habitats (Johnson et al., 2007), suggesting that the biological soil crusts may export more inorganic nitrogen to the soils below than gaseous losses to volatilization. Closed, saturated experimental conditions might reduce rapid oxidation, but would be an unrealistic condition that does not capture the potential dynamic of amoebae outcompeting nematodes in smaller pore spaces as the soil dries. Continued work is necessary to devise experimental systems that compare the relative role of microfaunal functional groups in realistic population sizes while limiting the adverse consequences of artificial conditions.

References

- Anderson, R. V., Elliott, E. T., McClellan, J. F., Coleman, D. C., Cole, C. V., & Hunt, H. W. (1978). Trophic interactions in soils as they affect energy and nutrient dynamics. III. Biotic interactions of bacteria, amoebae, and nematodes. *Microbial Ecology*, 4, 361-371.

- Beare, M. H., Parmelee, R. W., Hendrix, P. F., Cheng, W., Coleman, D. C., & Crossley, D. A. (1992). Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. *Ecological Monographs*, 62(4), 569-591.
- Cole, L., Dromph, K. M., Boaglio, V., & Bardgett, R. D. (2004). Effect of density and species richness of soil mesofauna on nutrient mineralisation and plant growth. *Biology and Fertility of Soils*, 39(5), 337-343.
- Darby, B. J., Housman, D. C., Zaki, A. M., Shamout, Y., Adl, S. M., Belnap, J., et al. (2006). Effects of altered temperature and precipitation on desert protozoa associated with biological soil crusts. *Journal of Eukaryotic Microbiology*, 53(6), 507-514.
- de Ruiter, P. C., van Veen, J. A., Moore, J. C., Brussaard, L., & Hunt, H. W. (1993). Calculation of nitrogen mineralization in soil food webs. *Plant and Soil*, 157, 263-273.
- Elliott, E. T., Anderson, R. V., Coleman, D. C., & Cole, C. V. (1980). Habitable pore space and microbial trophic interactions. *Oikos*, 35, 327-335.
- Hunt, H. W., Coleman, D. C., Ingham, E. R., Ingham, R. E., Elliott, E. T., Moore, J. C., et al. (1987). The detrital food web in a shortgrass prairie. *Biology and Fertility of Soils*, 3, 57-68.
- Ingham, E. R., & Coleman, D. C. (1984). Effects of streptomycin, cycloheximide, fungizone, captan, carbofuran, cygon, and PCNB on soil-microorganisms. *Microbial Ecology*, 10(4), 345-358.
- Ingham, E. R., Coleman, D. C., & Moore, J. C. (1989). An analysis of food-web structure and function in a shortgrass prairie, a mountain meadow, and a lodgepole pine forest. *Biology and Fertility of Soils*, 8(1), 29-37.
- Johnson, S. L., Budinoff, C. R., Belnap, J., & Garcia-Pichel, F. (2005). Relevance of ammonium oxidation within biological soil crust communities. *Environmental Microbiology*, 7(1), 1-12.
- Johnson, S. L., Neuer, S., & Garcia-Pichel, F. (2007). Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. *Environmental Microbiology*, 9(3), 680 to 689.
- Myrold, D. D., & Tiedje, J. M. (1986). Simultaneous estimation of several nitrogen-cycle rates using N-15 - theory and application. *Soil Biology & Biochemistry*, 18(6), 559-568.
- Saetre, P., & Stark, J. M. (2005). Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. *Oecologia*, 142(2), 247-260.

Table 30. Analysis of Microfauna from Nematode Addition Experiment.

A) F-values from analysis of \log_{10} to transformed biomass ($\mu\text{g dry wt. g}^{-1}$) of amoebae, flagellates, ciliates, and nematodes, and B) least-squares means and standard error of mean (SE) of time effect for \log_{10} to transformed biomass of microfauna.

A)	<u>Effect</u>	<u>df</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Time	2	28.26*	115.77***	11.46***	1.12
	Temp	1	0.22	12.64***	0.94	0.23
	Time*Temp	2	0.59	3.20*	3.73*	0.64
	Innoc	1	0.16	0.40	1.59	1.81
	Time*Innoc	2	3.47*	0.82	1.45	0.49
	Temp*Innoc	1	0.26	0.11	0.10	0.00
	Time*Temp*Innoc	2	0.54	0.59	1.17	0.00
	Residual:Block†		0.0	0.0	0.0070	0.021

B)	<u>Time</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Week 0	2.59 ^A	1.19 ^A	2.19 ^A	2.77
	Week 4	3.00 ^B	1.99 ^B	2.55 ^B	2.84
	Week 8	3.08 ^B	2.02 ^B	2.38 ^B	2.89
	(SE)	0.060	0.053	0.082	0.084

†Ratio of Residual:Block covariance parameters. Small values represent ineffective blocking that partitions little of the covariance, large values (near or above 1) represent effective blocking.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column) indicate contrasting means within an effect ($p < 0.05$). Means without a letter indicate a non-significant effect and no comparisons were made.

Table 31. Analysis of Inorganic Nitrogen from Nematode Addition Experiment.

A) F-values from analysis of \log_{10} to transformed concentration of ammonium and nitrate ($\mu\text{mol kg}^{-1}$), and B) least-squares means and standard error of mean (SE) of time effect for \log_{10} to transformed concentration of inorganic nitrogen.

A)	<u>Effect</u>	<u>df</u>	<u>NH₄</u>	<u>NO₃</u>
	Time	2	15.62***	2554.26***
	Temp	1	3.43	2.44
	Time*Temp	2	3.48*	1.45
	Innoc	1	6.78*	0.00
	Time*Innoc	2	3.62*	0.06
	Temp*Innoc	1	2.94	0.15
	Time*Temp*Innoc	2	1.01	0.55
	Residual:Block†		0.014	0.00023

B)	<u>Time</u>	<u>NH₄</u>	<u>NO₃</u>
	Week 0	0.92 ^A	1.82 ^A
	Week 4	0.13 ^C	3.03 ^B
	Week 8	0.48 ^B	3.21 ^C
	(SE)	0.143	0.016

†Ratio of Residual:Block covariance parameters. Small values represent ineffective blocking that partitions little of the covariance, large values (near or above 1) represent effective blocking.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column) indicate contrasting means within an effect ($p < 0.05$). Means without a letter indicate a non-significant effect and no comparisons were made.

Table 32. Analysis of Microfauna from Nematode Removal Experiment

A) F-values from analysis of \log_{10} to transformed biomass ($\mu\text{g dry wt. g}^{-1}$) of amoebae, flagellates, ciliates, and nematodes, and B) least-squares means and standard error of mean (SE) of time effect for \log_{10} to transformed biomass of microfauna.

A)	<u>Effect</u>	<u>df</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Time	2	56.42***	137.69***	4.26*	84.89***
	Temp	1	0.20	0.52	21.08***	15.97***
	Time*Temp	2	0.25	1.72	6.59**	7.57**
	Treat	1	1.02	0.26	0.40	68.08***
	Time*Treat	2	1.11	1.46	0.87	6.16**
	Temp*Treat	1	0.33	0.02	0.24	0.48
	Time*Temp*Treat	2	0.38	0.45	0.24	0.52
	Residual:Block†		0.0	0.022	0.020	8.55

B)	<u>Time</u>	<u>Amoebae</u>	<u>Flagellates</u>	<u>Ciliates</u>	<u>Nematodes</u>
	Week 0	2.71 ^A	1.09 ^A	2.28 ^A	2.79 ^A
	Week 4	2.89 ^B	1.67 ^B	2.04 ^B	2.65 ^B
	Week 8	3.44 ^C	2.16 ^C	2.26 ^A	2.45 ^C
	(SE)	0.057	0.056	0.072	0.126

†Ratio of Residual:Block covariance parameters. Small values represent ineffective blocking that partitions little of the covariance, large values (near or above 1) represent effective blocking.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column) indicate contrasting means within an effect ($p < 0.05$).

Table 33. Analysis of Inorganic Nitrogen from Nematode Removal Experiment.

A) F-values from analysis of \log_{10} to transformed concentration of ammonium and nitrate ($\mu\text{mol kg}^{-1}$), and B) least-squares means and standard error of mean (SE) of time effect for \log_{10} to transformed concentration of inorganic nitrogen.

A)	<u>Effect</u>	<u>df</u>	<u>NH₄</u>	<u>NO₃</u>
	Time	2	86.21***	1862.53***
	Temp	1	0.77	2.62
	Time*Temp	2	0.27	0.84
	Treat	1	0.13	2.39
	Time*Treat	2	0.92	0.17
	Temp*Treat	1	0.11	0.42
	Time*Temp*Treat	2	0.24	0.35
	Residual:Block†		0.34	1.44

B)	<u>Cover</u>	<u>NH₄</u>	<u>NO₃</u>
	Week 0	1.75 ^A	2.30 ^A
	Week 4	1.40 ^B	3.04 ^B
	Week 8	0.95 ^C	3.16 ^C
	(SE)	0.080	0.034

†Ratio of Residual:Block covariance parameters. Small values represent ineffective blocking that partitions little of the covariance, large values (near or above 1) represent effective blocking.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means with contrasting letter (within a column) indicate contrasting means within an effect ($p < 0.05$). Means without a letter indicate a non-significant effect and no comparisons were made.

Figure 26. Nematode Biomass and Inorganic Nitrogen from Nematode Addition Experiment.

Values represent nematode biomass ($\mu\text{g kg}^{-1}$) or inorganic nitrogen concentration ($\mu\text{mol kg}^{-1}$) through time under 26 °C day/16 °C night conditions (left side) or 36 °C day/26 °C night conditions (right side) and with (solid lines) or without (dashed lines) an inoculant of nematodes in addition to field abundance.

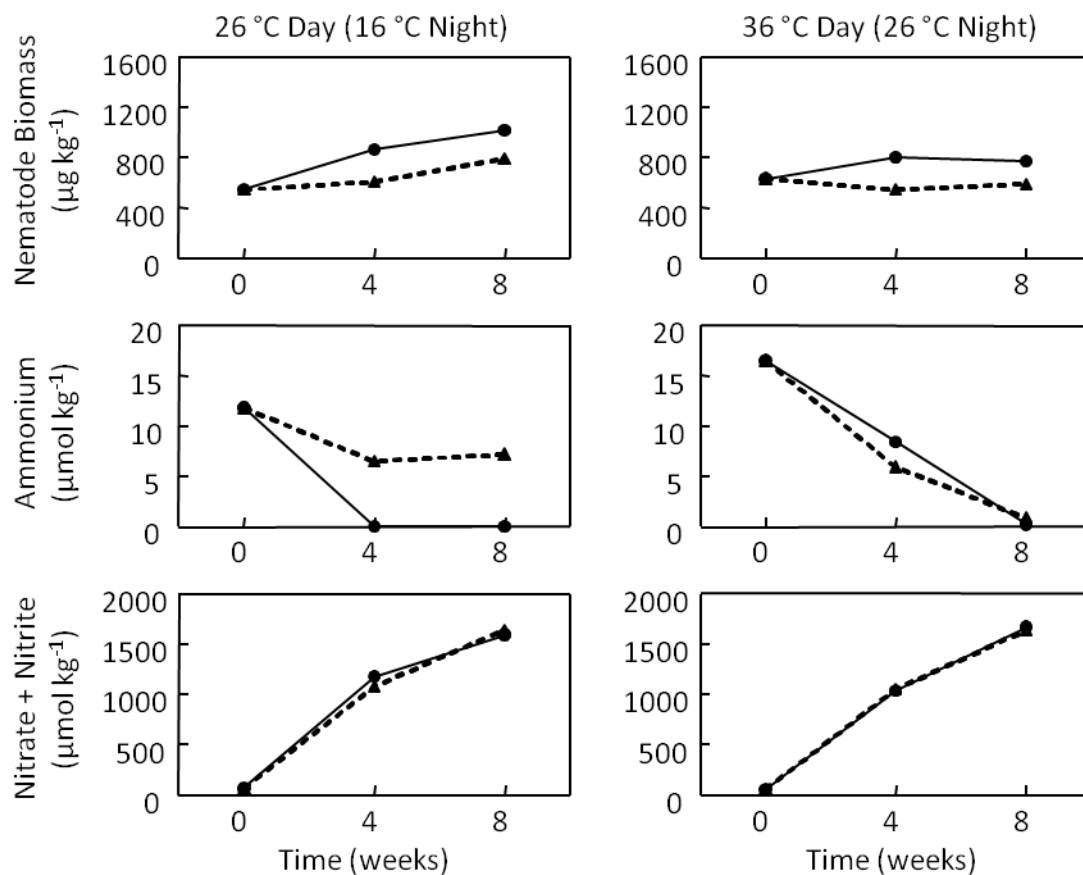


Figure 27. Protozoan Biomass from Nematode Addition Experiment.

Values represent biomass ($\mu\text{g kg}^{-1}$) through time under 26 °C day/16 °C night conditions (left side) or 36 °C day/26 °C night conditions (right side) and with (solid lines) or without (dashed lines) an inoculant of nematodes in addition to field abundance.

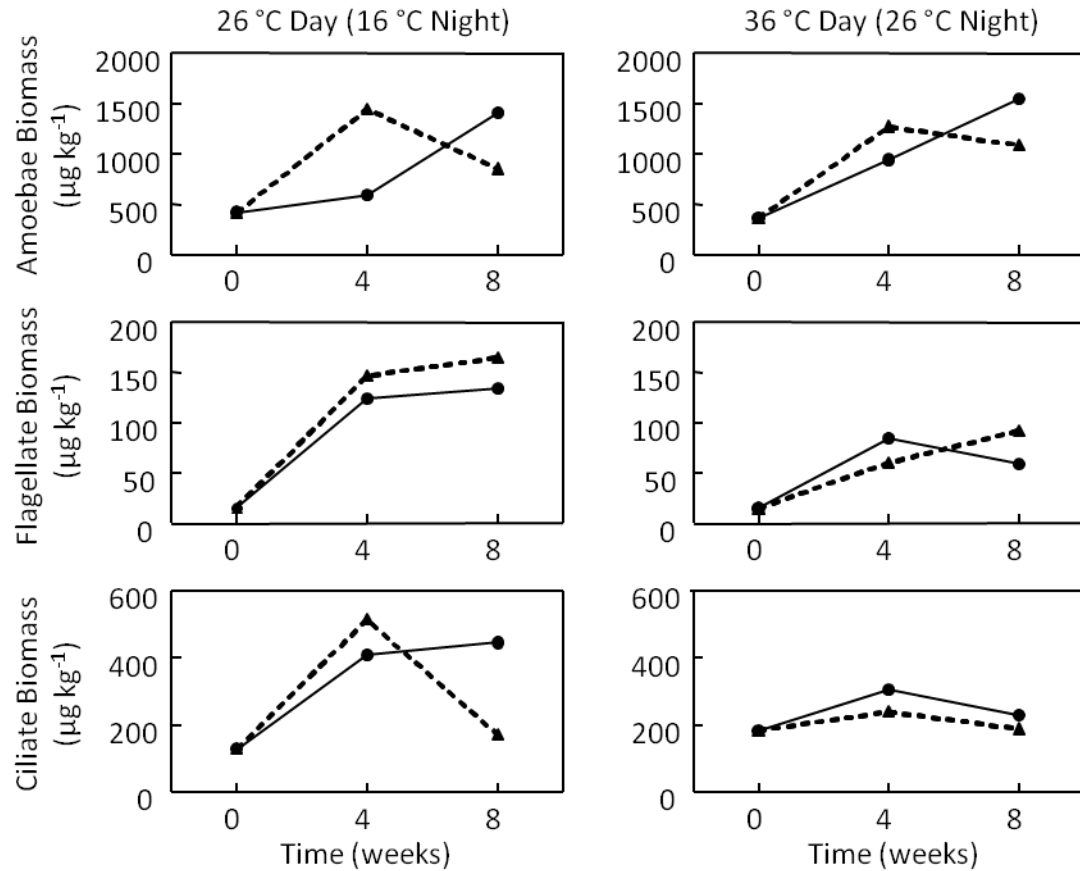


Figure 28. Nematode Biomass and Inorganic Nitrogen from Nematode Removal Experiment.

Values represent nematode biomass ($\mu\text{g kg}^{-1}$) or inorganic nitrogen concentration ($\mu\text{mol kg}^{-1}$) through time under 26 °C (left side) or 36 °C (right side) conditions and with (solid lines) or without (dashed lines) partial defaunation (45%) of nematodes with 48 hr at 65 °C.

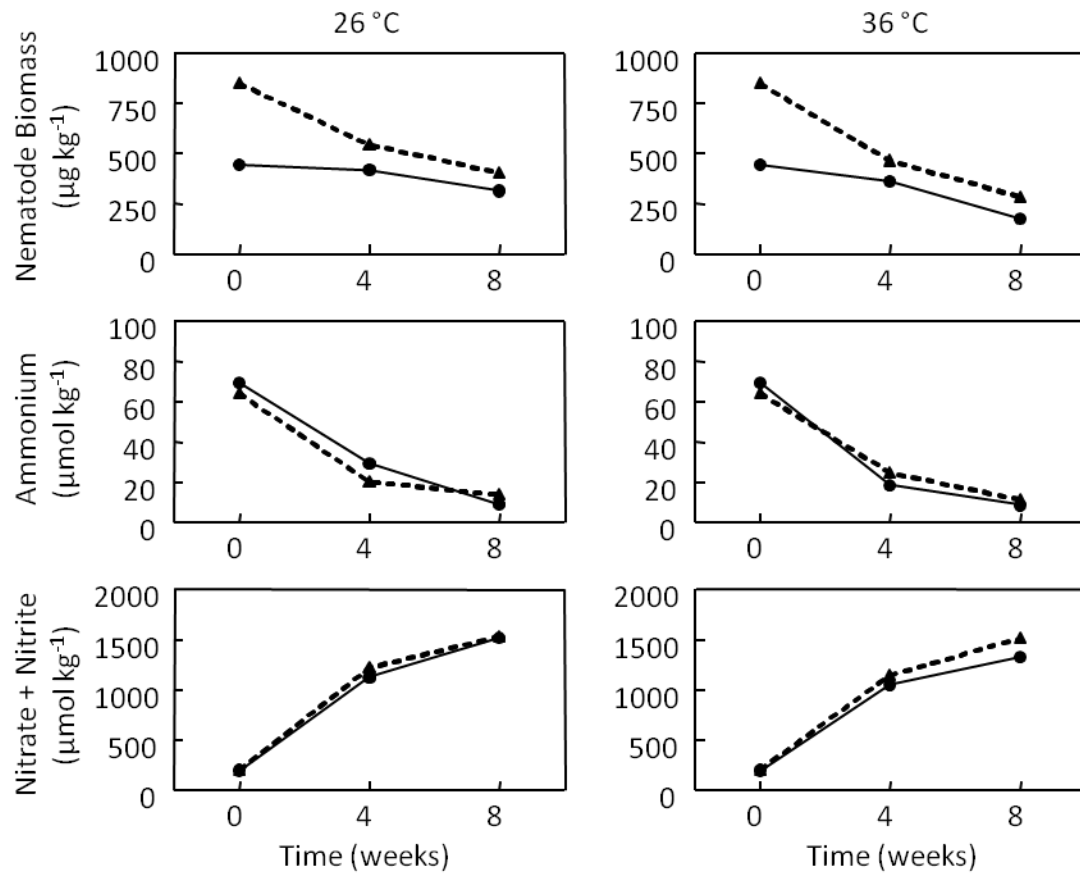
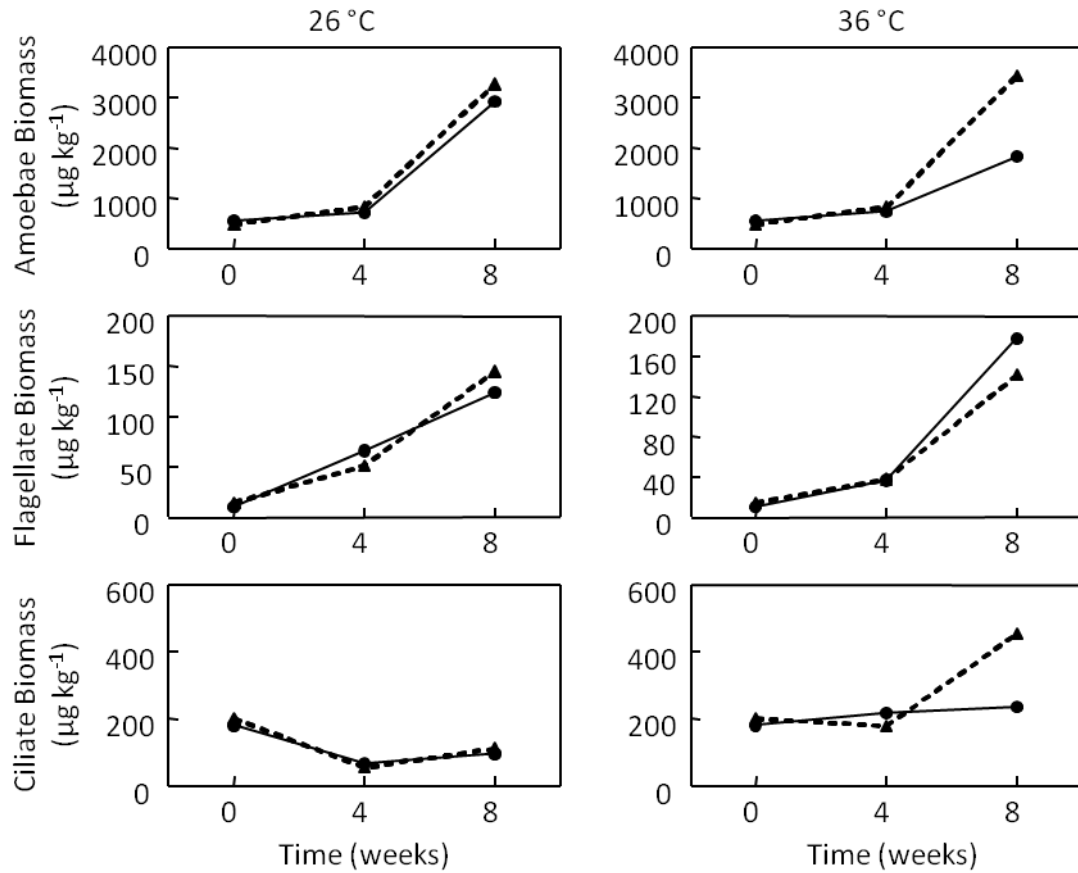


Figure 29. Protozoan Biomass from Nematode Removal Experiment.

Values represent biomass ($\mu\text{g kg}^{-1}$) through time under 26 °C (left side) or 36 °C (right side) conditions and with (solid lines) or without (dashed lines) partial defaunation (45%) of nematodes with 48 hr at 65 °C.



CHAPTER 8. COMPREHENSIVE BIBLIOGRAPHY

- Allen, M. W., & Noffsinger, E. M. (1971). *Nothacrobeles*, n. gen., with descriptions of four new species (Nematoda: Cephalobidae). *Journal of Nematology*, 3(2), 145-153.
- Alon, A., & Steinberger, Y. (1999). Response of the soil microbial biomass and nematode population to a wetting event in nitrogen-amended Negev Desert plots. *Biology and Fertility of Soils*, 30(1-2), 147-152.
- Anderson, R. V., & Coleman, D. C. (1982). Nematode temperature responses - a niche dimension in populations of bacterial-feeding nematodes. *Journal of Nematology*, 14(1), 69-76.
- Anderson, R. V., Elliott, E. T., McClellan, J. F., Coleman, D. C., Cole, C. V., & Hunt, H. W. (1978). Trophic interactions in soils as they affect energy and nutrient dynamics. III. Biotic interactions of bacteria, amoebae, and nematodes. *Microbial Ecology*, 4, 361-371.
- Anderson, R. V., Gould, W. D., Woods, L. E., Cambardella, C., Ingham, R. E., & Coleman, D. C. (1983). Organic and inorganic nitrogenous losses by microbivorous nematodes in soil. *Oikos*, 40, 75-80.
- Andrássy, I. (1956). Die rauminhalts- und gewichtsbestimmung der fadenwürmer (nematoden). *Acta Zoologica Hungarica*, 2, 1-15.
- Aseri, G. K., & Tarafdar, J. C. (2006). Fluorescein diacetate: A potential biological indicator for arid soils. *Arid Land Research and Management*, 20(2), 87-99.
- Ayres, E., Wall, D. H., Simmons, B. L., Field, C. B., Milchunas, D. G., Morgan, J. A., et al. (2008). Belowground nematode herbivores are resistant to elevated atmospheric CO₂ concentrations in grassland ecosystems. *Soil Biology & Biochemistry*, 40(4), 978-985.
- Bakonyi, G., & Nagy, P. (2000). Temperature- and moisture-induced changes in the structure of the nematode fauna of a semiarid grassland - patterns and mechanisms. *Global Change Biology*, 6(6), 697-707.
- Baldwin, J. G., De Ley, I. T., Mundo-Ocampo, M., De Ley, P., Nadler, S. A., & Gebre, M. (2001). *Acromoldavicus mojaviensis* n. sp. (Nematoda : Cephalobidae) from the Mojave Desert, California. *Nematology*, 3, 343-353.
- Bamforth, S. S. (1984). Microbial distributions in Arizona deserts and woodlands. *Soil Biology & Biochemistry*, 16(2), 133-137.
- Bamforth, S. S. (2004). Water film fauna of microbiotic crusts of a warm desert. *Journal of Arid Environments*, 56(3), 413-423.
- Bamforth, S. S. (2008). Protozoa of biological soil crusts of a cool desert in Utah. *Journal of Arid Environments*, 72(5), 722-729.
- Bazzaz, F. A. (1990). The response of natural ecosystems to the rising global CO₂ levels. *Annual Review of Ecology and Systematics*, 21, 167-196.

- Beare, M. H., Parmelee, R. W., Hendrix, P. F., Cheng, W., Coleman, D. C., & Crossley, D. A. (1992). Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. *Ecological Monographs*, 62(4), 569-591.
- Belnap, J. (1995). Surface disturbances: Their role in accelerating desertification. *Environmental Monitoring and Assessment*, 37(1-3), 39-57.
- Belnap, J. (2002). Impacts of off road vehicles on nitrogen cycles in biological soil crusts: Resistance in different US deserts. *Journal of Arid Environments*, 52(2), 155-165.
- Belnap, J. (2002). Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biology and Fertility of Soils*, 35(2), 128-135.
- Belnap, J. (2003). The world at your feet: Desert biological soil crusts. *Frontiers in Ecology and the Environment*, 1(4), 181-189.
- Belnap, J., & Gillette, D. A. (1997). Disturbance of biological soil crusts: Impacts on potential wind erodibility of sandy desert soils in southeastern Utah. *Land Degradation & Development*, 8(4), 355-362.
- Belnap, J., & Gillette, D. A. (1998). Vulnerability of desert biological soil crusts to wind erosion: The influences of crust development, soil texture, and disturbance. *Journal of Arid Environments*, 39(2), 133-142.
- Belnap, J., & Phillips, S. L. (2001). Soil biota in an ungrazed grassland: Response to annual grass (*Bromus tectorum*) invasion. *Ecological Applications*, 11(5), 1261-1275.
- Belnap, J., & Warren, S. D. (2002). Patton's tracks in the Mojave Desert, USA: An ecological legacy. *Arid Land Research and Management*, 16(3), 245-258.
- Belnap, J., Phillips, S. L., & Miller, M. E. (2004). Response of desert biological soil crusts to alterations in precipitation frequency. *Oecologia*, 141(2), 306-316.
- Belnap, J., Phillips, S. L., & Troxler, T. (2006). Soil lichen and moss cover and species richness can be highly dynamic: The effects of invasion by the annual exotic grass *Bromus tectorum*, precipitation, and temperature on biological soil crusts in SE Utah. *Applied Soil Ecology*, 32(1), 63-76.
- Belnap, J., Phillips, S. L., Sherrod, S. K., & Moldenke, A. (2005). Soil biota can change after exotic plant invasion: Does this affect ecosystem processes? *Ecology*, 86(11), 3007-3017.
- Benaroudj, N., Lee, D. H., & Goldberg, A. L. (2001). Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals. *Journal of Biological Chemistry*, 276(26), 24261-24267.
- Beymer, R. J., & Klopatek, J. M. (1991). Potential contribution of carbon by microphytic crusts in pinyon-juniper woodlands. *Arid Soil Research and Rehabilitation*, 5(3), 187-198.
- Billings, S. A., Schaeffer, S. M., & Evans, R. D. (2003). Nitrogen fixation by biological soil crusts and heterotrophic bacteria in an intact Mojave Desert ecosystem with elevated CO₂ and added soil carbon. *Soil Biology & Biochemistry*, 35(5), 643-649.

- Birkemoe, T., & Liengen, T. (2000). Does collembolan grazing influence nitrogen fixation by cyanobacteria in the high arctic? *Polar Biology*, 23(8), 589-592.
- Bongers, T. (1990). The maturity index: An ecological measure of environmental disturbance based on nematode species composition. *Oecologia*, 83, 14-19.
- Bongers, T. (1994). *De nematoden van nederland*. Utrecht, The Netherlands: Koninklijke Nedelandse Natuuistorische Vereniging.
- Bowker, M. A., Belnap, J., Davidson, D. W., & Phillips, S. L. (2005). Evidence for micronutrient limitation of biological soil crusts: Importance to arid-lands restoration. *Ecological Applications*, 15(6), 1941-1951.
- Bridgham, S. D., Pastor, J., Updegraff, K., Malterer, T. J., Johnson, K., Harth, C., et al. (1999). Ecosystem control over temperature and energy flux in northern peatlands. *Ecological Applications*, 9(4), 1345-1358.
- Carta, L. K., & Carta, D. G. (2000). Nematode specific gravity profiles and applications to flotation extraction and taxonomy. *Nematology*, 2, 201-210.
- Cochran, W. G. (1950). Estimation of bacterial densities by means of the "most probable number". *Biometrics*, 6, 105-116.
- Cole, L., Dromph, K. M., Boaglio, V., & Bardgett, R. D. (2004). Effect of density and species richness of soil mesofauna on nutrient mineralisation and plant growth. *Biology and Fertility of Soils*, 39(5), 337-343.
- Convey, P., & Wynn-Williams, D. D. (2002). Antarctic soil nematode response to artificial climate amelioration. *European Journal of Soil Biology*, 38(3-4), 255-259.
- Cookson, W. R., Muller, C., O'brien, P. A., Murphy, D. V., & Grierson, P. F. (2006). Nitrogen dynamics in an Australian semiarid grassland soil. *Ecology*, 87(8), 2047-2057.
- Crowe, J. H. (1971). Anhydrobiosis: An unsolved problem. *The American Naturalist*, 105(946), 563-573.
- Crowe, J. H., & Crowe, L. M. (1982). Induction of anhydrobiosis - membrane-changes during drying. *Cryobiology*, 19(3), 317-328.
- Crowe, J. H., & Madin, K. A. C. (1975). Anhydrobiosis in nematodes: Evaporative water loss and survival. *Journal of Experimental Zoology*, 193, 323-334.
- Crowe, J. H., Madin, K. A. C., & Loomis, S. H. (1977). Anhydrobiosis in nematodes: Metabolism during resumption of activity. *Journal of Experimental Zoology*, 201, 57-64.
- Darby, B. J., Housman, D. C., Zaki, A. M., Shamout, Y., Adl, S. M., Belnap, J., et al. (2006). Effects of altered temperature and precipitation on desert protozoa associated with biological soil crusts. *Journal of Eukaryotic Microbiology*, 53(6), 507-514.
- Darby, B. J., Neher, D. A., & Belnap, J. (2007). Soil nematode communities are ecologically more mature beneath late- than early-successional stage biological soil crusts. *Applied Soil Ecology*, 35(1), 203-212.

- Darbyshire, J. F., Wheatley, R. E., Greaves, M. P., & Inkson, R. H. E. (1974). A rapid micromethod for estimating bacteria and protozoan populations in soil. *Revue D Ecologie Et De Biologie Du Sol*, 11, 465-475.
- De Ley, I. T., De Ley, P., Baldwin, J. G., Mundo-Ocampo, M., & Nadler, S. A. (1999). Three new species of *Nothacrobeles* (Nemata: Cephalobidae) from the Mojave Desert, California. *Journal of Nematology*, 31(4), 482-497.
- De Ley, P. (1992). The nematode community of a marginal soil at Cambérène, Senegal, with special attention to functional morphology and niche partitioning in the family Cephalobidae. *Mededelingen van de Koninklijke Academie voor Wetenschappen, Letteren en Schone Kunsten van België - Klasse der Wetenschappen*, 53, 108-153.
- De Ley, P., De Ley, I. T., Mundo-Ocampo, M., Mundo, L., & Baldwin, J. (2003). *Identification of freeliving nematodes (secernentea)*. Riverside, California: University of California Extension.
- de Ruiter, P. C., van Veen, J. A., Moore, J. C., Brussaard, L., & Hunt, H. W. (1993). Calculation of nitrogen mineralization in soil food webs. *Plant and Soil*, 157, 263-273.
- DeMeure, Y., Freckman, D. W., & Van Gundy, S. D. (1979). Anhydrobiotic coiling of nematodes in soil. *Journal of Nematology*, 11(2), 189-195.
- Demeure, Y., Freckman, D. W., & Van Gundy, S. D. (1979). *In vitro* response of four species of nematodes to desiccation and discussion of this and related phenomena. *Revue de Nématologie*, 2(2), 203-210.
- Dodds, W. K., Gudder, D. A., & Mollenhauer, D. (1995). The ecology of *Nostoc*. *Journal of Phycology*, 31(1), 2-18.
- Duniway, J. M. (1976). Movement of zoospores of *Phytophthora cryptogea* in soils of various textures and matric potentials. *Phytopathology*, 66(7), 877-883.
- Easterling, D. R., Horton, B., Jones, P. D., Peterson, T. C., Karl, T. R., Parker, D. E., et al. (1997). Maximum and minimum temperature trends for the globe. *Science*, 277(5324), 364-367.
- Elliott, E. T., Anderson, R. V., Coleman, D. C., & Cole, C. V. (1980). Habitable pore space and microbial trophic interactions. *Oikos*, 35, 327-335.
- Evans, R. D., & Belnap, J. (1999). Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. *Ecology*, 80(1), 150 to 160.
- Evans, R. D., & Johansen, J. R. (1999). Microbiotic crusts and ecosystem processes. *Critical Reviews in Plant Sciences*, 18(2), 183-225.
- Ferris, H., Bongers, T., & De Goede, R. G. M. (2001). A framework for soil food web diagnostics: Extension of the nematode faunal analysis concept. *Applied Soil Ecology*, 18(1), 13-29.
- Fierer, N., & Schimel, J. P. (2003). A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Science Society of America Journal*, 67(3), 798-805.

- Foissner, W. (1999). Soil protozoa as bioindicators: Pros and cons, methods, diversity, representative examples. *Agriculture Ecosystems & Environment*, 74(1-3), 95-112.
- Freckman, D. W. (1983). Survival mechanisms in nematodes. *Journal of Nematology*, 15(4), 479.
- Freckman, D. W., & Mankau, R. (1986). Abundance, distribution, biomass and energetics of soil nematodes in a northern Mojave Desert ecosystem. *Pedobiologia*, 29(2), 129-142.
- Freckman, D. W., Kaplan, D. T., & van Gundy, S. D. (1977). A comparison of techniques for extraction and study of anhydrobiotic nematodes from dry soils. *Journal of Nematology*, 9(2), 176-181.
- Freckman, D. W., Whitford, W. G., & Steinberger, Y. (1987). Effect of irrigation on nematode population-dynamics and activity in desert soils. *Biology and Fertility of Soils*, 3(1-2), 3-10.
- George, D. B., Roundy, B. A., St Clair, L. L., Johansen, J. R., Schaalje, G. B., & Webb, B. L. (2003). The effects of microbiotic soil crusts on soil water loss. *Arid Land Research and Management*, 17(2), 113-125.
- Green, V. S., Stott, D. E., & Diack, M. (2006). Assay for fluorescein diacetate hydrolytic activity: Optimization for soil samples. *Soil Biology & Biochemistry*, 38(4), 693-701.
- Griffiths, B. S., Young, I. M., & Caul, S. (1995). Nematode and protozoan population-dynamics on decomposing barley leaves incubated at different soil matrix potentials. *Pedobiologia*, 39(5), 454-461.
- Guppy, M., & Withers, P. (1999). Metabolic depression in animals: Physiological perspectives and biochemical generalizations. *Biological Reviews*, 74(1), 1-40.
- Hand, S. C., & Hardewig, I. (1996). Downregulation of cellular metabolism during environmental stress: Mechanisms and implications. *Annual Review of Physiology*, 58, 539-563.
- Harte, J., Torn, M. S., Chang, F. R., Feifarek, B., Kinzig, A. P., Shaw, R., et al. (1995). Global warming and soil microclimate - results from a meadow-warming experiment. *Ecological Applications*, 5(1), 132-150.
- Hawkes, C. V. (2003). Nitrogen cycling mediated by biological soil crusts and arbuscular mycorrhizal fungi. *Ecology*, 84(6), 1553-1562.
- Hawkins, A. J. S. (1987). Thermal responses reflect protein turnover in *Mytilus edulis* L. *Functional Ecology*, 1(4), 339-351.
- Hawkins, A. J. S. (1991). Protein-turnover - a functional appraisal. *Functional Ecology*, 5(2), 222-233.
- Hendrix, P. F., Parmelee, R. W., Crossley, D. A., Jr., Coleman, D. C., Odum, E. P., & Groffman, P. M. (1986). Detritus food webs in conventional and no-tillage agroecosystems. *BioScience*, 36(6), 374-380.

- Housman, D. C., Naumburg, E., Huxman, T. E., Charlet, T. N., Nowak, R. S., & Smith, S. D. (2006). Increases in desert shrub productivity under elevated carbon dioxide vary with water availability. *Ecosystems*, 9(3), 374-385.
- Housman, D. C., Yeager, C. M., Darby, B. J., Sanford, R. L., Kuske, C. R., Neher, D. A., et al. (2007). Heterogeneity of soil nutrients and subsurface biota in a dryland ecosystem. *Soil Biology & Biochemistry*, 39(8), 2138-2149.
- Hu, C. X., Liu, Y. D., Song, L. R., & Zhang, D. K. (2002). Effect of desert soil algae on the stabilization of fine sands. *Journal of Applied Phycology*, 14(4), 281-292.
- Hubert, J., & Lukesova, A. (2001). Feeding of the panphytophagous oribatid mite *Scheloribates laevigatus* (Acari : Oribatida) on cyanobacterial and algal diets in laboratory experiments. *Applied Soil Ecology*, 16(1), 77-83.
- Hunt, D. J. (1993). Aphelenchida, Longidoridae and Trichodoridae: Their systematics and bionomics. Cambridge, UK: CAB International.
- Hunt, H. W., & Wall, D. H. (2002). Modelling the effects of loss of soil biodiversity on ecosystem function. *Global Change Biology*, 8(1), 33-50.
- Hunt, H. W., Coleman, D. C., Ingham, E. R., Ingham, R. E., Elliott, E. T., Moore, J. C., et al. (1987). The detrital food web in a shortgrass prairie. *Biology and Fertility of Soils*, 3, 57-68.
- Ingham, E. R., & Coleman, D. C. (1984). Effects of streptomycin, cycloheximide, fungizone, captan, carbofuran, cygon, and PCNB on soil-microorganisms. *Microbial Ecology*, 10(4), 345-358.
- Ingham, E. R., Coleman, D. C., & Moore, J. C. (1989). An analysis of food-web structure and function in a shortgrass prairie, a mountain meadow, and a lodgepole pine forest. *Biology and Fertility of Soils*, 8(1), 29-37.
- Ingham, R. E., Trofymow, J. A., Ingham, E. R., & Coleman, D. C. (1985). Interactions of bacteria, fungi, and their nematode grazers: Effects on nutrient cycling and plant growth. *Ecol. Mono.*, 55(1), 119-140.
- Jairajpuri, M. S., & Ahmad, W. (1992). Dorylaimida: Free-living, predaceous and plant-parasitic nematodes. New York: E.J. Brill.
- Jeffries, D. L., Klopatek, J. M., Link, S. O., & Bolton, H. (1992). Acetylene-reduction by cryptogamic crusts from a blackbrush community as related to resaturation and dehydration. *Soil Biology & Biochemistry*, 24(11), 1101-1105.
- Jenkins, W. R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter*, 48(9), 692.
- Johnson, S. L., Budinoff, C. R., Belnap, J., & Garcia-Pichel, F. (2005). Relevance of ammonium oxidation within biological soil crust communities. *Environmental Microbiology*, 7(1), 1-12.
- Johnson, S. L., Neuer, S., & Garcia-Pichel, F. (2007). Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. *Environmental Microbiology*, 9(3), 680 to 689.
- Jones, K. L., Todd, T. C., Wall-Beam, J. L., Coolon, J. D., Blair, J. M., & Herman, M. A. (2006). Molecular approach for assessing responses of microbial-feeding

- nematodes to burning and chronic nitrogen enrichment in a native grassland. *Molecular Ecology*, 15(9), 2601-2609.
- Jordan, D. N., Zitzer, S. F., Hendrey, G. R., Lewin, K. F., Nagy, J., Nowak, R. S., et al. (1999). Biotic, abiotic and performance aspects of the Nevada Desert Free-Air CO₂ enrichment (FACE) facility. *Global Change Biology*, 5(6), 659-668.
- Khan, Z., Park, S. D., Shin, S. Y., Bae, S. G., Yeon, I. K., & Seo, Y. J. (2005). Management of *Meloidogyne incognita* on tomato by root-dip treatment in culture filtrate of the blue-green alga, *Microcoleus vaginatus*. *Bioresource Technology*, 96(12), 1338-1341.
- Leibold, M. A. (1995). The niche concept revisited: Mechanistic models and community context. *Ecology*, 76(5), 1371-1382.
- Liang, W., & Steinberger, Y. (2001). Temporal changes in nematode community structure in a desert ecosystem. *Journal of Arid Environments*, 48(3), 267-280.
- Lide, D. (2001-2002). Handbook for chemistry and physics, 82nd edition. New York: CRC Press.
- Littell, R. C., Milliken, G. A., Stroup, W. W., & Wolfinger, R. D. (1996). *SAS system for mixed models*. Cary, NC: SAS Institute, Inc.
- Logan, J. A., Wollkind, D. J., Hoyt, S. C., & Tanigoshi, L. K. (1976). An analytic model for description of temperature dependent rate phenomena in arthropods. *Environmental Entomology*, 5(6), 1133-1140.
- Loreau, M., Mouquet, N., & Gonzalez, A. (2003). Biodiversity as spatial insurance in heterogeneous landscapes. *Proceedings of the National Academy of Sciences of the United States of America*, 100(22), 12765-12770.
- Madin, K. A. C., Loomis, S. H., & Crowe, J. H. (1985). Anhydrobiosis in nematodes - control of carbon flow through the glyoxylate cycle. *Journal of Experimental Zoology*, 234(3), 341-350.
- Marchant, R., & Nicholas, W. L. (1974). An energy budget for the free-living nematode *Pelodera* (rhabditidae). *Oecologia*, 16, 237-252.
- Melillo, J. M., McGuire, A. D., Kicklighter, D. W., Moore, B., Vorosmarty, C. J., & Schloss, A. L. (1993). Global climate-change and terrestrial net primary production. *Nature*, 363(6426), 234-240.
- Millbank, J. W. (1982). The assessment of nitrogen-fixation and throughput by lichens .3. Losses of nitrogenous compounds by *Peltigera membranacea*, *Peltigera polydactyla* and *Lobaria pulmonaria* in simulated rainfall episodes. *New Phytologist*, 92(2), 229-234.
- Moore, J. C., & de Ruiter, P. C. (1991). Temporal and spatial heterogeneity of trophic interactions within below-ground food webs. *Agriculture, Ecosystems and Environment*, 34, 371-397.
- Myrold, D. D., & Tiedje, J. M. (1986). Simultaneous estimation of several nitrogen-cycle rates using N-15 - theory and application. *Soil Biology & Biochemistry*, 18(6), 559-568.

- Neher, D. A., Walters, T. L., Tramer, E., Weicht, T. R., Veluci, R. M., Saiya-Cork, K., et al. (2003). Biological soil crust and vascular plant communities in a sand savanna of northwestern ohio. *Journal of the Torrey Botanical Society*, 130(4), 244-252.
- Neher, D. A., Weicht, T. R., Moorhead, D. L., & Sinsabaugh, R. L. (2004). Elevated CO₂ alters functional attributes of nematode communities in forest soils. *Functional Ecology*, 18(4), 584-591.
- Nowak, M. A., & Sigmund, K. (2004). Evolutionary dynamics of biological games. *Science*, 303(5659), 793-799.
- Osler, G. H. R., & Sommerkorn, M. (2007). Toward a complete soil C and N cycle: Incorporating the soil fauna. *Ecology*, 88(7), 1611-1621.
- Page, F. C. (1988). *A new key to freshwater and soil gymnamoebae*. Ambleside, Cumbria, UK: Freshwater Biological Association.
- Parker, L. W., Freckman, D. W., Steinberger, Y., Driggers, L., & Whitford, W. G. (1984). Effects of simulated rainfall and litter quantities on desert soil biota - soil respiration, microflora, and protozoa. *Pedobiologia*, 27(3), 185-195.
- Parker, L. W., Santos, P. F., Phillips, J., & Whitford, W. G. (1984). Carbon and nitrogen dynamics during the decomposition of litter and roots of a Chihuahuan Desert annual, *Lepidium lasiocarpum*. *Ecological Monographs*, 54(3), 339-360.
- Peterjohn, W. T., & Schlesinger, W. H. (1990). Nitrogen loss from deserts in the southwestern United-States. *Biogeochemistry*, 10(1), 67-79.
- Peterjohn, W. T., & Schlesinger, W. H. (1991). Factors controlling denitrification in a Chihuahuan Desert ecosystem. *Soil Science Society of America Journal*, 55(6), 1694-1701.
- Petersen, H., & Luxton, M. (1982). A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos*, 39, 288-388.
- Reynolds, R. L., Belnap, J., Reheis, M., Lamothe, P., & Luiszer, F. (2001). Aeolian dust in Colorado Plateau soils: Nutrient inputs and recent change in source. *Proceedings of the National Academy of Sciences*, 98, 7123-7127.
- Ricci, C., & Pagani, M. (1997). Desiccation of *Panagrolaimus rigidus* (Nematoda): Survival, reproduction and the influence on the internal clock. *Hydrobiologia*, 347, 1-13.
- Ricci, C., & Perletti, F. (2006). Starve and survive: Stress tolerance and life-history traits of a bdelloid rotifer. *Functional Ecology*, 20(2), 340 to 346.
- Ricci, C., Vaghi, L., & Manzini, M. L. (1987). Desiccation of rotifers (*Macrotrachela quadricornifera*) - survival and reproduction. *Ecology*, 68(5), 1488-1494.
- Robinson, A. F. (1995). Optimal release rates for attracting *Meloidogyne incognita*, *Rotylenchulus reniformis*, and other nematodes to carbon-dioxide in sand. *Journal of Nematology*, 27(1), 42-50.
- Robinson, A. F., & Heald, C. M. (1991). Carbon-dioxide and temperature-gradients in Baermann funnel extraction of *Rotylenchulus reniformis*. *Journal of Nematology*, 23(1), 28-38.

- Robinson, A. F., Orr, C. C., & Heintz, C. E. (1981). Effects of oxygen and temperature on the activity and survival of *Nothanguina phyllobia*. *Journal of Nematology*, 13(4), 528-535.
- Robinson, B. S., Bamforth, S. S., & Dobson, P. J. (2002). Density and diversity of protozoa in some arid australian soils. *Journal of Eukaryotic Microbiology*, 49(6), 449-453.
- Rodriguez-Zaragoza, S., Mayzlish, E., & Steinberger, Y. (2005). Vertical distribution of the free-living amoeba population in soil under desert shrubs in the Negev Desert, Israel. *Applied and Environmental Microbiology*, 71(4), 2053-2060.
- Rogerson, A., & Patterson, D. J. (2000). The naked ramicristate amoebae (Gymnamoebae). In J. J. Lee, G. F. Leedale & P. Bradbury (Eds.), *The illustrated guide to the protozoa*, 2nd ed. (pp. 1023-1052). Lawrence: Allen Press.
- Rosentreter, R., & Belnap, J. (2001). Biological soil crusts of North America. In J. Belnap & O. L. Lange (Eds.), *Biological soil crusts: Structure, function, and management* (pp. 31-50). New York: Springer-Verlag.
- Rychert, R. C., & Skujins, J. (1974). Nitrogen fixation by blue-green algae-lichen crusts in the Great Basin Desert. *Soil Science Society of America Proceedings*, 38, 768-771.
- Rychert, R. C., Skujins, J., Sorensen, D., & Porcella, D. (1978). Nitrogen fixation by lichens and free-living microorganisms in deserts. In N. E. West & J. Skujins (Eds.), *Nitrogen in desert ecosystems* (pp. 20 to 30). Stroudsburg: Dowden, Hutchinson and Ross, Inc.
- Ryszkowski, L. (1975). Energy and matter economy of ecosystems. In W. H. van Dobben & R. H. Lowe-McConnel (Eds.), *Unifying concepts in ecology* (pp. 109-126). The Hague: Junk.
- Saetre, P., & Stark, J. M. (2005). Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. *Oecologia*, 142(2), 247-260.
- Santos, P. F., & Whitford, W. G. (1981). The effects of microarthropods on litter decomposition in a Chihuahuan Desert ecosystem. *Ecology*, 62(3), 654-663.
- Santos, P. F., Phillips, J., & Whitford, W. G. (1981). The role of mites and nematodes in early stages of buried litter decomposition in a desert. *Ecology*, 62(3), 664-669.
- Schimel, J. P., & Bennett, J. (2004). Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*, 85(3), 591-602.
- Schlesinger, W. H., & Peterjohn, W. T. (1991). Processes controlling ammonia volatilization from Chihuahuan Desert soils. *Soil Biology & Biochemistry*, 23(7), 637-642.
- Schlesinger, W. H., & Pilmanis, A. M. (1998). Plant-soil interactions in deserts. *Biogeochemistry*, 42(1-2), 169-187.
- Schlesinger, W. H., Raikes, J. A., Hartley, A. E., & Cross, A. E. (1996). On the spatial pattern of soil nutrients in desert ecosystems. *Ecology*, 77(2), 364-374.

- Schlesinger, W. H., Reynolds, J. F., Cunningham, G. L., Huenneke, L. F., Jarrell, W. M., Virginia, R. A., et al. (1990). Biological feedbacks in global desertification. *Science*, 247(4946), 1043-1048.
- Seager, R., Ting, M. F., Held, I., Kushnir, Y., Lu, J., Vecchi, G., et al. (2007). Model projections of an imminent transition to a more arid climate in southwestern North America. *Science*, 316(5828), 1181-1184.
- Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, 27, 379-423, 623-656.
- Siddiqi, M. R. (2000). *Tylenchida: Parasites of plants and insects*, 2nd ed. Cambridge, UK: CAB International.
- Singh, B. N. (1946). A method of estimating the numbers of soil protozoa, especially amoebae based on their differential feeding on bacteria. *Annals of Applied Biology*, 33, 112-119.
- Smirnov, A. V., & Brown, S. (2004). Guide to the methods of study and identification of soil gymnamoebae. *Protistology*, 3(3), 148-190.
- Smith, S. D., Huxman, T. E., Zitzer, S. F., Charlet, T. N., Housman, D. C., Coleman, J. S., et al. (2000). Elevated CO₂ increases productivity and invasive species success in an arid ecosystem. *Nature*, 408(6808), 79-82.
- Sohlenius, B., & Bostrom, S. (1999). Effects of climate change on soil factors and metazoan microfauna (nematodes, tardigrades and rotifers) in a Swedish tundra soil - a soil transplantation experiment. *Applied Soil Ecology*, 12(2), 113-128.
- Sorensen, J. G., Kristensen, T. N., & Loeschke, V. (2003). The evolutionary and ecological role of heat shock proteins. *Ecology Letters*, 6(11), 1025-1037.
- Steinberger, Y., & Loboda, I. (1991). Nematode population dynamics and trophic structure in a soil profile under the canopy of the desert shrub *Zygophyllum dumosum*. *Pedobiologia*, 35(3), 191-197.
- Steinberger, Y., & Sarig, S. (1993). Response to soil nematode populations and the soil microbial biomass to a rain episode in the hot, dry Negev Desert. *Biol. Fertil. Soils*, 16, 188-192.
- Steinberger, Y., Freckman, D. W., Parker, L. W., & Whitford, W. G. (1984). Effects of simulated rainfall and litter quantities on desert soil biota - nematodes and microarthropods. *Pedobiologia*, 26(4), 267-274.
- Steinberger, Y., Liang, W. J., Savkina, E., Meshi, T., & Barnes, G. (2001). Nematode community composition and diversity associated with a topoclimatic transect in a rain shadow desert. *European Journal of Soil Biology*, 37(4), 315-320.
- Stenchikov, G. L., & Robock, A. (1995). Diurnal asymmetry of climatic response to increased CO₂ and aerosols: Forcings and feedbacks. *Journal of Geophysical Research-Atmospheres*, 100(D12), 26211-26227.
- Stone, D. A., & Weaver, A. J. (2002). Daily maximum and minimum temperature trends in a climate model. *Geophysical Research Letters*, 29(9).
- Thorne, G. (1925). The genus *Acrobeles* von Linstow, 1887. *Transactions of American Microscopical Society*, 44(4), 171-210.

- Thorne, G. (1937). A revision of the nematode family Cephalobidae, Chitwood and Chitwood, 1934. *Proceedings of the Helminthological Society of Washington*, 4(1), 1-16.
- Thorne, G. (1974). *Nematodes of the Northern Great Plains. Part I. Tylenchida (Nemata: Secernentea)*. Brookings, SD: Tech. Bull. 41, Agricultural Experiment Station, South Dakota State University.
- Thorne, G. (1974). *Nematodes of the Northern Great Plains. Part II. Dorylaimoidea in part (Nemata: Adenophorea)*. Brookings, SD: Tech. Bull. 41, Agricultural Experiment Station, South Dakota State University.
- Todd, T. C., Blair, J. M., & Milliken, G. A. (1999). Effects of altered soil-water availability on a tallgrass prairie nematode community. *Applied Soil Ecology*, 13(1), 45-55.
- Treonis, A. M., Wall, D. H., & Virginia, R. A. (2000). The use of anhydrobiosis by soil nematodes in the Antarctic dry valleys. *Functional Ecology*, 14(4), 460 to 467.
- Van den Brink, P. J., & Ter Braak, C. J. F. (1998). Multivariate analysis of stress in experimental systems by principal response curves and similarity analysis. *Aquatic Ecology*, 32, 163-178.
- Van den Brink, P. J., & Ter Braak, C. J. F. (1998). Multivariate analysis of stress in experimental systems by principal response curves and similarity analysis. *Aquatic Ecology*, 32, 163-178.
- Van Den Brink, P. J., & Ter Braak, C. J. F. (1999). Principal Response Curves: Analysis of time-dependent multivariate responses of biological community to stress. *Environmental Toxicology and Chemistry*, 18(2), 138-148.
- Viglierchio, D. R., & Schmitt, R. V. (1983). On the methodology of nematode extraction from field samples - Baermann funnel modifications. *Journal of Nematology*, 15(3), 438-444.
- Viglierchio, D. R., & Schmitt, R. V. (1983). On the methodology of nematode extraction from field samples - comparison of methods for soil extraction. *Journal of Nematology*, 15(3), 450 to 454.
- Viglierchio, D. R., & Yamashita, T. T. (1983). On the methodology of nematode extraction from field samples - density flotation techniques. *Journal of Nematology*, 15(3), 444-449.
- Walters, C., Hill, L. M., & Wheeler, L. J. (2005). Dying while dry: Kinetics and mechanisms of deterioration in desiccated organisms. *Integrative and Comparative Biology*, 45(5), 751-758.
- Weltzin, J. F., Loik, M. E., Schwinning, S., Williams, D. G., Fay, P. A., Haddad, B. M., et al. (2003). Assessing the response of terrestrial ecosystems to potential changes in precipitation. *Bioscience*, 53(10), 941-952.
- West, G. B., Brown, J. H., & Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science*, 276(5309), 122-126.
- West, G. B., Brown, J. H., & Enquist, B. J. (1999). The fourth dimension of life: Fractal geometry and allometric scaling of organisms. *Science*, 284(5420), 1677-1679.

- West, N. E., & Skujins, J. (1977). The nitrogen cycle in North American cold-winter semi-desert ecosystems. *Ecologia Plantarum*, 12, 45-53.
- Westh, P., & Ramlov, H. (1991). Trehalose accumulation in the tardigrade *Adorybiotus coronifer* during anhydrobiosis. *Journal of Experimental Zoology*, 258(3), 303-311.
- Wharton, D. A. (2003). The environmental physiology of antarctic terrestrial nematodes: A review. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, 173(8), 621-628.
- Whitehead, A. G., & Hemming, J. R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, 55, 25-38.
- Whitford, W. G., Aldon, E. F., Freckman, D. W., Steinberger, Y., & Parker, L. W. (1989). Effects of organic amendments on soil biota on a degraded rangeland. *Journal of Range Management*, 42(1), 56-60.
- Whitford, W. G., Freckman, D. W., Elkins, N. Z., Parker, L. W., Parmalee, R., Phillips, J., et al. (1981). Diurnal migration and responses to simulated rainfall in desert soil microarthropods and nematodes. *Soil Biology & Biochemistry*, 13(5), 417-425.
- Whitford, W. G., Steinberger, Y., Mackay, W., Parker, L. W., Freckman, D., Wallwork, J. A., et al. (1986). Rainfall and decomposition in the Chihuahuan Desert. *Oecologia*, 68(4), 512-515.
- Williams, J. D., Dobrowolski, J. P., West, N. E., & Gillette, D. A. (1995). Microphytic crust influence on wind erosion. *Transactions of the ASAE*, 38(1), 131-137.
- Wood, F. H. (1973). Biology of *Aporcelaimellus* sp. (Nematoda: Aporcelaimidae). *Nematologica*, 19, 528-537.
- Wood, F. H. (1973). Biology of *Aporcelaimellus* sp. (Nematoda: Aporcelaimidae). *Nematologica*, 19, 528-537.
- Wood, F. H. (1973). Feeding relationships of soil-dwelling nematodes. *Soil Biol. Biochem.*, 5, 593-601.
- Wright, D. J. (1975). Elimination of nitrogenous compounds by *Panagrellus redivivus*, Goodey, 1945 (Nematoda: Cephalobidae). *Comparative Biochemistry and Physiology. B, Comparative Biochemistry*, 52B, 247-253.
- Wright, D. J. (1975). Studies on nitrogen catabolism in *Panagrellus redivivus*, Goodey, 1945 (Nematoda: Cephalobidae). *Comparative Biochemistry and Physiology. B, Comparative Biochemistry*, 52B, 255-260.
- Wright, P. A. (1995). Nitrogen excretion: Three end products, many physiological roles. *Journal of Experimental Biology*, 198(2), 273-281.
- Yamaoka, M., Wato, T., & Matsuoka, T. (2004). Effects of salt concentration and bacteria on encystment induction in ciliated protozoan *Colpoda* sp. *Acta Protozoologica*, 43(2), 93-98.
- Yeates, G. W. (1994). Modification and qualification of the nematode maturity index. *Pedobiologia*, 38, 97-101.

- Yeates, G. W., Bongers, T., de Goede, R. G. M., Freckman, D. W., & Georgieva, S. S. (1993). Feeding habits in soil nematode families and genera - an outline for soil ecologists. *J. Nematology*, 25(3), 315-331.
- Zaragoza, S. R., & Steinberger, Y. (2004). Seasonal dynamics of amoebae in the root canopy of *Zygophyllum dumosum* in the Negev Desert, Israel. *Pedobiologia*, 48(3), 277-281.
- Zavaleta, E. S., Shaw, M. R., Chiariello, N. R., Thomas, B. D., Cleland, E. E., Field, C. B., et al. (2003). Grassland responses to three years of elevated temperature, CO₂, precipitation, and N deposition. *Ecological Monographs*, 73(4), 585-604.
- Zheng, D. W., Agren, G. I., & Bengtsson, J. (1999). How do soil organisms affect total organic nitrogen storage and substrate nitrogen to carbon ratio in soils? A theoretical analysis. *Oikos*, 86(3), 430 to 442.